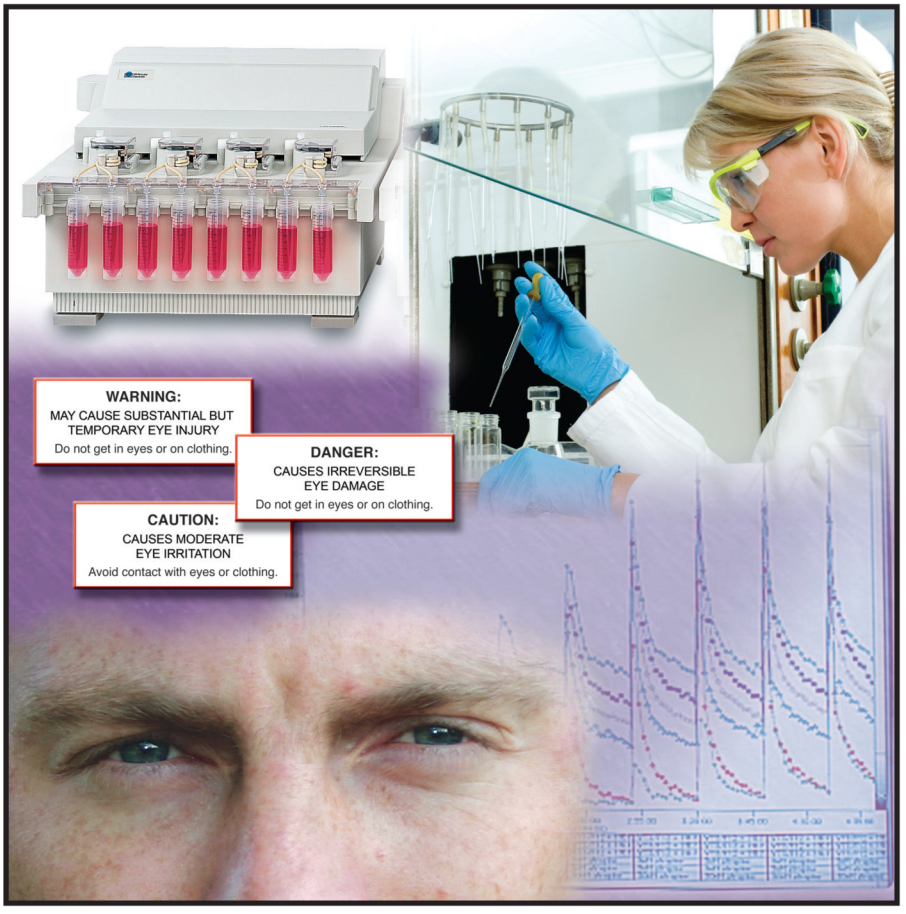
NIH Publication Number 10-7553A



ICCVAM Test Method Evaluation Report: Current Validation Status of *In Vitro* Test Methods Proposed for Identifying Eye Injury Hazard Potential of Chemicals and Products

Volume 1 of 2

Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)

National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

National Institute of Environmental Health Sciences
National Institutes of Health
U.S. Public Health Service
Department of Health and Human Services

About the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

In 1997, the National Institute of Environmental Health Sciences (NIEHS), one of the National Institutes of Health, established ICCVAM to:

- Coordinate interagency technical reviews of new and revised toxicological test methods, including alternative test methods that reduce, refine, or replace the use of animals
- Coordinate cross-agency issues relating to validation, acceptance, and national and international harmonization of new, modified, and alternative toxicological test methods

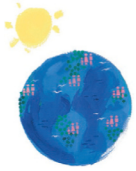
On December 19, 2000, the ICCVAM Authorization Act (Public Law 106-545, 42 U.S.C. 285*l*-3) established ICCVAM as a permanent interagency committee of NIEHS under NICEATM.

ICCVAM conducts technical evaluations of new, revised, and alternative methods with regulatory applicability. ICCVAM promotes the scientific validation and regulatory acceptance of toxicological test methods that more accurately assess the safety or hazards of chemicals and products and that reduce, refine (decrease or eliminate pain and distress), and/or replace animal use. NICEATM administers ICCVAM and provides scientific and operational support for ICCVAM-related activities. More information about NICEATM and ICCVAM can be found on the NICEATM-ICCVAM website (http://iccvam.niehs.nih.gov) or obtained by contacting NICEATM (telephone: [919] 541-2384, e-mail: niceatm@niehs.nih.gov).

ICCVAM is an interagency committee with representatives from the following 15 U.S. Federal regulatory and research member agencies that require, use, generate, or disseminate toxicological information:*

- *Consumer Product Safety Commission*
- *Department of Agriculture*
- *Department of Defense*
- *Department of Energy*
- Department of Health and Human Services
  - Centers for Disease Control and Prevention
    - *Agency for Toxic Substances and Disease Registry*
    - *National Institute of Occupational Safety and Health*
  - *Food and Drug Administration*
  - National Institutes of Health
    - *Office of the Director*
    - *National Cancer Institute*
    - *National Institute of Environmental Health Sciences*
    - *National Library of Medicine*
- *Department of the Interior*
- Department of Labor
  - *Occupational Safety and Health Administration*
- *Department of Transportation*
- *Environmental Protection Agency*

*Italics indicate those agencies represented on ICCVAM, as specified in the ICCVAM Authorization Act.



The NICEATM-ICCVAM earth-and-sun graphic symbolizes the important role of new and alternative toxicological methods in protecting and advancing the health of people, animals, and our environment.

On the cover: This collage of images representing in vitro *test methods proposed for identifying the eye injury hazard potential of test substances includes (clockwise from top left): apparatus used to conduct the Cytosensor Microphysiometer test method; a scientist wearing protective eyewear while working in the laboratory; a graphic representation of data from the Cytosensor Microphysiometer test method; human eyes to represent the importance of prevention of eye injuries; examples of warning labels that might appear on containers of chemicals or products with the potential to cause eye injury. (Cytosensor Microphysiometer images courtesy of Molecular Devices, Inc.)*

ICCVAM Test Method Evaluation Report: Current Validation Status of *In Vitro* Test Methods Proposed for Identifying Eye Injury Hazard Potential of Chemicals and Products

Volume 1 of 2

Interagency Coordinating Committee on the Validation of Alternative Methods

National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods

National Institute of Environmental Health Sciences
National Institutes of Health
U.S. Public Health Service
Department of Health and Human Services

2010

NIH Publication Number 10-7553A

National Toxicology Program
P.O. Box 12233
Research Triangle Park, NC 27709

This document is available electronically at:
http://iccvam.niehs.nih.gov/methods/ocutox/MildMod-TMER.htm

When referencing this document, please cite as follows:
ICCVAM. 2010. ICCVAM Test Method Evaluation Report: Current Validation Status of *In Vitro* Test Methods Proposed for Identifying Eye Injury Hazard Potential of Chemicals and Products. NIH Publication No. 10-7553. Research Triangle Park, NC: National Institute of Environmental Health Sciences.

Table of Contents
Volume 1 of 2

List of Tables viii

List of Abbreviations and Acronyms ix

Interagency Coordinating Committee on the Validation of Alternative Methods: Agency Representatives x

Acknowledgements xi

Preface xvii

Executive Summary xix

1.0 Introduction 1

2.0 The Bovine Corneal Opacity and Permeability Test Method 3

2.1 ICCVAM Recommendations 3

2.1.1 ICCVAM Recommendations: Test Method Usefulness and Limitations 3

2.1.2 ICCVAM Recommendations: BCOP Test Method Protocol 6

2.1.3 ICCVAM Recommendations: Future Studies for the BCOP Test Method 6

2.1.4 ICCVAM Recommendations: Performance Standards for the BCOP Test Method 7

2.2 Validation Status of the BCOP Test Method 7

2.2.1 Test Method Description 7

2.2.2 Validation Database 7

2.2.3 Test Method Accuracy 8

2.2.4 Test Method Reliability 10

2.2.5 Animal Welfare Considerations 11

3.0 The Cytosensor Microphysiometer Test Method 12

3.1 ICCVAM Recommendations 12

3.1.1 ICCVAM Recommendations: Test Method Usefulness and Limitations 12

3.1.2 ICCVAM Recommendations: CM Test Method Protocol 13

3.1.3 ICCVAM Recommendations: Future Studies for the CM Test Method 14

3.1.4 ICCVAM Recommendations: Performance Standards for the CM Test Method 14

3.2 Validation Status of the Cytosensor Microphysiometer Test Method 14

3.2.1 Test Method Description 15

3.2.2 Validation Database 15

3.2.3 Test Method Accuracy 15

3.2.4 Test Method Reliability 18

3.2.5 Animal Welfare Considerations 18

4.0 The Hen's Egg Test–Chorioallantoic Membrane Test Method 19

4.1 ICCVAM Recommendations 19

4.1.1 ICCVAM Recommendations: Test Method Usefulness and Limitations 19

4.1.2 ICCVAM Recommendations: HET-CAM Test Method Protocol 20

4.1.3 ICCVAM Recommendations: Future Studies for the HET-CAM Test Method 20

4.1.4 ICCVAM Recommendations: Performance Standards for the HET-CAM Test Method 21

4.2 Validation Status of the HET-CAM Test Method 21

4.2.1 Test Method Description 21

4.2.2 Validation Database 21

4.2.3 Test Method Accuracy 22

4.2.4 Test Method Reliability 24

4.2.5 Animal Welfare Considerations 25

5.0 The Isolated Chicken Eye Test Method 26

5.1 ICCVAM Recommendations 26

5.1.1 ICCVAM Recommendations: Test Method Usefulness and Limitations 26

5.1.2 ICCVAM Recommendations: ICE Test Method Protocol 27

5.1.3 ICCVAM Recommendations: Future Studies for the ICE Test Method 27

5.1.4 ICCVAM Recommendations: Performance Standards for the ICE Test Method 27

5.2 Validation Status of the ICE Test Method 28

5.2.1 Test Method Description 28

5.2.2 Validation Database 28

5.2.3 Test Method Accuracy 28

5.2.4 Test Method Reliability 30

5.2.5 Animal Welfare Considerations 31

6.0 The Isolated Rabbit Eye Test Method 32

6.1 ICCVAM Recommendations 32

6.1.1 ICCVAM Recommendations: Test Method Usefulness and Limitations 32

6.1.2 ICCVAM Recommendations: IRE Test Method Protocol 32

6.1.3 ICCVAM Recommendations: Future Studies for the IRE Test Method 33

6.1.4 ICCVAM Recommendations: Performance Standards for the IRE Test Method 33

6.2 Validation Status of the IRE Test Method 33

6.2.1 Test Method Description 34
6.2.2 Validation Database 34
6.2.3 Test Method Accuracy 34
6.2.4 Test Method Reliability 35
6.2.5 Animal Welfare Considerations 35

7.0 ICCVAM Consideration of Public, SACATM, and ICATM Comments 36

7.1 Public Comments in Response to 70 FR 13512 (March 21, 2005) Request for Data on Non-Animal Methods and Approaches for Determining Skin and Eye Irritation Potential of Antimicrobial Cleaning Product Formulations; Request for Nominations for an Independent Expert Panel 37

7.2 Public Comments in Response to 72 FR 26396 (May 9, 2007) Request for Data on the Use of Topical Anesthetics and Systemic Analgesics for *In Vivo* Eye Irritation Testing 37

7.3 Public Comments in Response to 72 FR 31582 (June 7, 2007) Request for Ocular Irritancy Test Data From Human, Rabbit, and *In Vitro* Studies Using Standardized Testing Methods 37

7.4 Public Comments in Response to 73 FR 18535 (April 4, 2008) Non-Animal Methods and Approach for Evaluating Eye Irritation Potential for Antimicrobial Cleaning Products (AMCPs): Request for Nominations for an Independent Expert Panel and Submission of Relevant Data 37

7.5 Public Comments in Response to 74 FR 14556 (March 31, 2009) Announcement of an Independent Scientific Peer Review Panel on Alternative Ocular Safety Testing Methods; Availability of Draft Background Review Documents (BRD); Request for Comments 38

7.6 Public Comments in Response to 74 FR 19562 (April 29, 2009) Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) 41

7.7 Public Comments in Response to 74 FR 33444 (July 13, 2009) Independent Scientific Peer Review Panel Report: Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches; Notice of Availability and Request for Public Comments 41

7.8 Comments Received from ICATM Validation Organizations 41

8.0 References 43

Appendix A ICCVAM Evaluation Timeline A-1

Appendix B ICCVAM-Recommended Test Method Protocols B-1

B1 ICCVAM-Recommended Protocol for Future Studies Using the Bovine Corneal Opacity And Permeability (BCOP) Test Method B-3

B2 ICCVAM-Recommended Protocol for Future Studies Using the Cytosensor Microphysiometer (CM) Test Method B-19

B3 ICCVAM-Recommended Protocol for Future Studies Using the Hen's Egg Test–Chorioallantoic Membrane (HET-CAM) Test Method B-29

B4 ICCVAM-Recommended Protocol for Future Studies Using the Isolated Chicken Eye (ICE) Test Method B-39

B5 ICCVAM-Recommended Protocol for Future Studies Using the Isolated Rabbit Eye (IRE) Test Method B-51

Appendix C Background Review Document – Current Status of *In Vitro* Test Methods for Identifying Mild/Moderate Ocular Irritants: Bovine Corneal Opacity and Permeability Test Method C-1

Appendix D Background Review Document of Existing Methods for Eye Irritation Testing: Silicon Microphysiometer and Cytosensor Microphysiometer D-1

Table of Contents
Volume 2 of 2

List of Tables ..... **viii**

List of Abbreviations and Acronyms ..... **ix**

Interagency Coordinating Committee on the Validation of Alternative Methods: Agency Representatives ..... **x**

Acknowledgements ..... **xi**

Preface ..... **xvii**

Executive Summary ..... **xix**

Appendix E **Background Review Document – Current Status of *In Vitro* Test Methods for Identifying Mild/Moderate Ocular Irritants: The Hen's Egg Test–Chorioallantoic Membrane (HET-CAM) Test Method** **E-1**

Appendix F **Background Review Document – Current Status of *In Vitro* Test Methods for Identifying Mild/Moderate Ocular Irritants: The Isolated Chicken Eye (ICE) Test Method** **F-1**

Appendix G **Independent Scientific Peer Review Panel Assessment** **G-1**

G1 Summary Minutes from the Peer Review Panel Meeting on May 19–21, 2009 ..... G-3

G2 Independent Scientific Peer Review Panel Report: Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches G-31

Appendix H ***Federal Register* Notices and Public Comments** **H-1**

H1 *Federal Register* Notices H-3

H2 Public Comments Received in Response to Federal Register Notices H-19

H3 Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) Comments: SACATM Meeting on June 25–26, 2009 ..... H-109

H4 ECVAM Comments on ICCVAM Recommendations for the BCOP Test Method H-119

Appendix I **Relevant U.S. Federal and International Ocular Toxicity Regulation, Labeling and Test Guidelines** **I-1**

Appendix J **NICEATM Analysis: Reduced Eye Hazard Labeling Resulting from Using Globally Harmonized System (GHS) Instead of Current U.S. Regulatory Classification Criteria** **J-1**

Appendix K **ECVAM Scientific Advisory Committee (ESAC) – Statement on the Scientific Validity of Cytotoxicity/Cell-Function Based *In Vitro* Assays for Eye Irritation Testing** **K-1**

List of Tables

Table 2-1 *In Vivo* Ocular Lesions from False Negative Substances in the BCOP Test Method Using the EPA Classification System 5

Table 2-2 Evaluation of the Performance of the BCOP Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by EPA, EU, and GHS Classification Systems 9

Table 2-3 Accuracy of the BCOP Test Method for Distinguishing Substances Not Labeled as Irritants from All Other Irritant Classes 10

Table 3-1 Accuracy of the CM Test Method for Distinguishing Substances Not Labeled as Irritants from All Other Irritant Classes for Surfactant-Containing Substances 16

Table 3-2 Accuracy of the CM Test Method for Distinguishing Substances Not Labeled as Irritants from All Other Irritant Classes for Nonsurfactant Substances 16

Table 3-3 Accuracy of the CM Test Method for Distinguishing Corrosives/Severe Irritants from All Other Irritant Classes for Surfactant-Containing Substances 17

Table 3-4 Accuracy of the CM Test Method for Distinguishing Corrosives/Severe Irritants from All Other Irritant Classes for Nonsurfactant Substances 18

Table 4-1 Evaluation of the Performance of the HET-CAM Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the EPA, EU, and GHS Classification Systems 23

Table 4-2 Accuracy of the HET-CAM Test Method for Distinguishing Substances Not Labeled as Irritants from All Other Irritant Classes 24

Table 5-1 Evaluation of the Performance of the ICE Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the EPA, EU, and GHS Classification Systems 29

Table 5-2 Accuracy of the ICE Test Method for Distinguishing Substances Not Labeled as Irritants from All Other Irritant Classes 30

Table 7-1 Opportunities for Public Comments 36

List of Abbreviations and Acronyms

AMCP	Antimicrobial cleaning product
BCOP	Bovine corneal opacity and permeability
BRD	Background review document
CAM	Chorioallantoic membrane
CEC	Commission of European Communities
CM	Cytosensor® Microphysiometer
CV	Coefficient of variation
ºC	Degrees centigrade
ECVAM	European Centre for the Validation of Alternative Methods
EPA	U.S. Environmental Protection Agency
ESAC	ECVAM Scientific Advisory Committee
EU	European Union
FHSA	Federal Hazardous Substances Act
FR	*Federal Register*
g	Gram
GHS	United Nations Globally Harmonized System of Classification and Labelling of Chemicals
HET-CAM	Hen's egg test–chorioallantoic membrane
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ICE	Isolated chicken eye
IRE	Isolated rabbit eye
IS	Irritation score
MeSH	Medical Subject Headings
mL	Milliliter
NICEATM	National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
NTP	U.S. National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OTWG	ICCVAM Ocular Toxicity Working Group
SACATM	Scientific Advisory Committee on Alternative Toxicological Methods
UN	United Nations
UV/VIS	Ultraviolet/Visible

Interagency Coordinating Committee on the Validation of Alternative Methods: Agency Representatives

Agency for Toxic Substances and Disease Registry
* Moiz Mumtaz, Ph.D.
Bruce Fowler, Ph.D.
Edward Murray, Ph.D.
Eric Sampson, Ph.D.

Consumer Product Safety Commission
* Marilyn L. Wind, Ph.D. (Chair)
+ Kristina Hatlelid, Ph.D.
Joanna Matheson, Ph.D.

Department of Agriculture
* Jodie Kulpa-Eddy, D.V.M. (Vice-Chair)
+ Elizabeth Goldentyer, D.V.M.

Department of Defense
* Robert E. Foster, Ph.D.
+ Patty Decot
Harry Salem, Ph.D.
Peter J. Schultheiss, D.V.M., DACLAM

Department of Energy
* Michael Kuperberg, Ph.D.
+ Marvin Stodolsky, Ph.D.

Department of the Interior
* Barnett A. Rattner, Ph.D.
+ Sarah Gerould, Ph.D. (to Feb. 2009)

Department of Transportation
* George Cushmac, Ph.D.
+ Steve Hwang, Ph.D.

Environmental Protection Agency
Office of Pesticide Programs
* John R. "Jack" Fowle III, Ph.D., DABT
+ Vicki Dellarco, Ph.D.
+ Tina Levine, Ph.D.
Deborah McCall
Christine Augustyniak, Ph.D. *(U.S. Coordinator, OECD Test Guidelines Program)*
Office of Pollution Prevention and Toxics
Jerry Smrchek, Ph.D. *(U.S. Coordinator, OECD Test Guidelines Program,* to July 2009*)*
Office of Research and Development
Suzanne McMaster, Ph.D. (to Dec. 2008)
Julian Preston, Ph.D. (to July 2009)
Stephanie Padilla, Ph.D. (to July 2009)
Office of Science Coordination and Policy
Karen Hamernik, Ph.D. (to July 2009)

Food and Drug Administration
Office of the Commissioner
* Suzanne Fitzpatrick, Ph.D., DABT
Center for Biologics Evaluation and Research
Richard McFarland, Ph.D., M.D.
Ying Huang, Ph.D.
Center for Devices and Radiological Health
Melvin E. Stratmeyer, Ph.D.
Vasant G. Malshet, Ph.D., DABT
Center for Drug Evaluation and Research
+ Abigail C. Jacobs, Ph.D.
Paul C. Brown, Ph.D.
Center for Food Safety and Applied Nutrition
David G. Hattan, Ph.D.
Robert L. Bronaugh, Ph.D.
Center for Veterinary Medicine
Devaraya Jagannath, Ph.D.
M. Cecilia Aguila, D.V.M.
National Center for Toxicological Research
Paul Howard, Ph.D.
Donna Mendrick, Ph.D.
William T. Allaben, Ph.D. (to Jan. 2009)
Office of Regulatory Affairs
Lawrence D'Hoostelaere, Ph.D.

National Cancer Institute
* T. Kevin Howcroft, Ph.D.
Chand Khanna, D.V.M., Ph.D.
Alan Poland, M.D. (to Oct. 2008)

National Institute of Environmental Health Sciences
* William S. Stokes, D.V.M., DACLAM
+ Raymond R. Tice, Ph.D.
Rajendra S. Chhabra, Ph.D., DABT
Jerrold J. Heindel, Ph.D.

National Institute for Occupational Safety and Health
* Paul Nicolaysen, V.M.D.
+ K. Murali Rao, M.D., Ph.D.

National Institutes of Health
* Margaret D. Snyder, Ph.D.

National Library of Medicine
* Pertti (Bert) Hakkinen, Ph.D.
+ Jeanne Goshorn, M.S.

Occupational Safety and Health Administration
* Surender Ahir, Ph.D.

* Principal agency representative
+ Alternate principal agency representative

Acknowledgements

Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Ocular Toxicity Working Group (OTWG)

U.S. Consumer Product Safety Commission
Marilyn L. Wind, Ph.D.
Adrienne Layton, Ph.D.

U.S. Department of Defense
Harry Salem, Ph.D.

U.S. Department of Transportation
Steve Hwang, Ph.D.

U.S. Environmental Protection Agency
Office of Pesticide Programs
Meta Bonner, Ph.D.
Jonathan Chen, Ph.D.
John R. "Jack" Fowle III, Ph.D., DABT
Masih Hashim, D.V.M., Ph.D.
Karen Hicks
Marianne Lewis
Debbie McCall
Timothy McMahon, Ph.D.
Mark Perry
John Redden
Jenny Tao, Ph.D.
Office of Research and Development
Andrew Geller, Ph.D.
Office of Science Coordination and Policy
Karen Hamernik, Ph.D.

U.S. Food and Drug Administration
Center for Drug Evaluation and Research
Paul Brown, Ph.D.
Wiley Chambers, M.D.
Abigail (Abby) Jacobs, Ph.D.
Jill Merrill, Ph.D., DABT (OTWG Chair)
Center for Food Safety and Applied Nutrition
Robert Bronaugh, Ph.D.
Donnie Lowther
Office of the Commissioner
Suzanne Fitzpatrick, Ph.D., DABT

National Institute of Environmental Health Sciences
Warren Casey, Ph.D., DABT
Mark F. Cesta, D.V.M, DACVP
Raymond (Buck) Grissom, Ph.D.
William Stokes, D.V.M., DACLAM

Occupational Safety and Health Administration
Surender Ahir, Ph.D.

European Centre for the Validation of Alternative Methods – Liaison
João Barroso, Ph.D.
Thomas Cole, Ph.D.
Valerie Zuang, Ph.D.

Japanese Center for the Validation of Alternative Methods – Liaison
Hajime Kojima, Ph.D.

Alternative Ocular Safety Testing Methods and Approaches Independent Scientific Peer Review Panel (May 19–21, 2009)

Hongshik Ahn, Ph.D.
Professor
Stony Brook University
Stony Brook, NY

Paul T. Bailey, Ph.D.
Bailey & Associates Consulting
Neshanic Station, NJ

Richard Dubielzig, D.V.M.
Professor
School of Veterinary Medicine
University of Wisconsin-Madison
Madison, WI

Henry Edelhauser, Ph.D.[1]
Professor of Ophthalmology and Director of Ophthalmic Research
Emory University School of Medicine
Atlanta, GA

Mark Evans, D.V.M., Ph.D., DACVP
Pathology Lead for Ophthalmology Therapeutic Area
Pfizer Global Research and Development
La Jolla Drug Safety Research and Development
San Diego, CA

A. Wallace Hayes, Ph.D., DABT, FATS, ERT
Visiting Scientist (Harvard)
Principal Advisor
Spherix Incorporated
Bethesda, MD
Harvard School of Public Health
Andover, MA

James V. Jester, Ph.D.
Professor of Ophthalmology and Biomedical Engineering
Endowed Chair
University of California-Irvine
Orange, CA

Tadashi Kosaka, D.V.M., Ph.D.
Associate Director
Chief, Laboratory of Immunotoxicology and Acute Toxicology
Toxicology Division
The Institute of Environmental Toxicology
Ibaraki, Japan

Alison McLaughlin, M.Sc., DABT
Health Canada
Environmental Impact Initiative
Office of Science and Risk Management
Health Products and Food Branch
Ottawa, Ontario, Canada

J. Lynn Palmer, Ph.D.
Associate Professor
Dept. of Palliative Care & Rehabilitation Medicine
University of Texas
MD Anderson Cancer Center
Houston, TX

Robert Peiffer, Jr., D.V.M., Ph.D., DACVO
Senior Investigator
Safety Assessment Toxicology
Merck Research Laboratories
Doylestown, PA

Denise Rodeheaver, Ph.D., DABT
Assistant Director
Dept. of Toxicology
Alcon Research Ltd.
Fort Worth, TX

Donald Sawyer, D.V.M., Ph.D., DACVA
Professor Emeritus
Retired, Michigan State University
(Summer Residence)
Okemos, MI
(Winter Residence)
Tucson, AZ

[1] Drs. Edelhauser, Thake, and Tseng were unable to attend the public meeting on May 19–21, 2009. However, they were involved in the peer review of the background review documents and concur with the conclusions and recommendations included in the *Independent Scientific Peer Review Panel Report – Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Strategies.*

Kirk Tarlo, Ph.D., DABT
Scientific Director
Comparative Biology and Safety Sciences
Amgen, Inc.
Thousand Oaks, CA

Daryl C. Thake, D.V.M., DACVP[1]
Midwest ToxPath Sciences Inc.
Chesterfield, MO

Scheffer Tseng, M.D., Ph.D.[1]
Director, Ocular Surface (OS) Center
Medical Director OS Research & Education Foundation
Director R&D Department
Tissue Tech, Inc.
Ocular Surface Center, P.A.
Miami, FL

Jan van der Valk, Ph.D.
Senior Scientist
Department of Animals in Science and Society
Faculty of Veterinary Medicine
Utrecht University
Netherlands Centre Alternatives to Animal Use
Utrecht, Netherlands

Philippe Vanparys, Ph.D., DABT
Managing Director
CARDAM: Centre for Advanced Research & Development
Mol, Belgium

Maria Pilar Vinardell, Ph.D.
Director, Department of Physiology
Professor of Physiology and Pathology
Department Fisologia
Facultat de Farmacia
Universitat de Barcelona
Barcelona, Spain

Fu-Shin Yu, Ph.D.
Director of Research
Department of Ophthalmology & Anatomy
School of Medicine
Wayne State University
Detroit, MI

Sherry Ward, Ph.D., MBA
In Vitro Toxicology Consultant
BioTred Solutions
Science Advisor
International Foundation for Ethical Research
New Market, MD

Daniel Wilson, Ph.D., DABT
Mammalian Toxicology Consultant, Toxicology and Environmental Research Consulting
The Dow Chemical Co.
Midland, MI

National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

National Institute of Environmental Health Sciences

William Stokes, D.V.M., DACLAM
Director; Project Officer

Warren Casey, Ph.D., DABT
Deputy Director

Deborah McCarley
Special Assistant; Assistant Project Officer

NICEATM Support Contract Staff (Integrated Laboratory Systems [ILS], Inc.)

David Allen, Ph.D.
Jonathan Hamm, Ph.D.
Nelson Johnson
Brett Jones, Ph.D.
Elizabeth Lipscomb, Ph.D.
Linda Litchfield
Steven Morefield, M.D.
Catherine Sprankle
James Truax, M.A.
Linda Wilson

Statistical Consultant for ILS, Inc.

Joseph Haseman, Ph.D.

Other Acknowledgements

ICCVAM and NICEATM gratefully acknowledge the following individuals and institutions that submitted data to NICEATM for the evaluation of alternative ocular safety testing methods and approaches.

Access Business Group
Luann Potts
Tom Truszkowski
Washington, DC

Clorox
Pleasanton, CA

Colgate-Palmolive Company
Piscataway, NJ

Cosmetics, Toiletry, and Fragrance Association
Carol Eisenmann, Ph.D.
Washington, DC

The Dial Corporation
Scottsdale, AZ

ECOLabs
St. Paul, MN

ECVAM
Chantra Eskes, Ph.D.
Ispra, Italy

ExxonMobil Biomedical Sciences, Inc.
James Freeman, Ph.D.
Annandale, NJ

Institute for In Vitro Sciences, Inc.
Rodger Curren, Ph.D.
John Harbell Ph.D. (until March 2006)
Jennifer Nash, M.S.
Angela Sizemore, B.S.
Gaithersburg, MD

JohnsonDiversey, Inc.
John Hamilton, Ph.D.
Sarah Willems, B.S.
Sturdivant, WI

Johnson & Johnson Pharmaceutical R&D
Phillipe Vanparys, Ph.D.
Freddy van Goethem, Ph.D.
Beerse, Belgium

L'Oreal
Christine Van den Berghe, Ph.D.
Paris, France

MatTek Corporation
Patrick Hayden, Ph.D.
Ashland. MA

Merck
Joseph Sina, Ph.D.
West Point, PA

National Institute of Health Sciences
Yasuo Ohno, Ph.D.
Tokyo, Japan

S.C. Johnson & Son
Nicole Cuellar, M.S.
Judith Swanson, B.S./B.A.
Racine, WI

The Procter & Gamble Company
Dan Marsman, D.V.M., Ph.D., DABT
Len Sauers, Ph.D.
Cincinnati, OH

TNO Nutrition and Food Research Institute
Menk Prinsen, Ph.D.
Zeist, The Netherlands

U.S. Food and Drug Administration
Donnie Lowther
College Park, MD

ZEBET
Manfred Liebsch, Ph.D.
Horst Spielmann, Dr. med.
Berlin, Germany

This page intentionally left blank

Preface

Eye injury is a leading cause of visual impairment in the United States with 40,000 to 50,000 new cases of impaired vision reported each year.[1] Many eye injuries occur due to contact with workplace or household products or chemicals. Accidents involving common household products (e.g., oven cleaner and bleach) cause about 125,000 eye injuries each year.[2] These products often cause chemical burns and emergency room visits.[3] Each day about 2,000 U.S. workers have a job-related eye injury that requires medical treatment. Although the majority of these eye injuries result from mechanical sources, chemical burns from industrial chemicals or cleaning products are common.[4]

To prevent eye injuries, regulatory agencies require testing to determine if chemicals and products may cause eye damage. This testing information is used to classify the ocular hazard and determine appropriate labeling to warn consumers and workers of the potential hazard. Appropriate labeling tells users how to avoid exposure that could damage the eye and what emergency procedures should be followed if there is accidental exposure. Nearly all ocular safety testing has been conducted using the Draize rabbit eye test (Draize et al. 1944), although *in vitro* methods can now be used to identify whether substances cause severe irritation or permanent eye damage. The Draize rabbit eye test involves instillation of 0.1 mL of the test substance into the conjunctival sac of one eye. The other eye serves as the untreated control. The eye is examined at least daily for up to 21 days. The presence and severity of any injuries to the cornea, conjunctiva, and the iris (tissues inside the eye) are scored, and the duration that the injuries persist is recorded.

In 2006, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) evaluated the validation status of the bovine corneal opacity and permeability (BCOP), hen's egg test–chorioallantoic membrane (HET-CAM), isolated chicken eye (ICE), and isolated rabbit eye (IRE) test methods for their ability to identify ocular corrosives and severe irritants. Based on the validation database and performance, ICCVAM recommended that positive results in the BCOP and ICE test methods could be used to identify ocular corrosives and severe irritants without the need for animal testing. These test methods should always be considered before using animals and should be used where determined appropriate. Following their acceptance by U.S. Federal regulatory agencies in 2008, the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and ICCVAM developed Organisation for Economic Co-operation and Development (OECD) international test guidelines for the BCOP and ICE test methods. The OECD adopted the guidelines in 2009.[5] As a result, substances that may cause severe irritation or permanent damage to eyes can now be identified using these methods without the use of live animals in the 31 member countries of the OECD.

This test method evaluation report provides ICCVAM's recommendations regarding the BCOP, HET-CAM, ICE, and IRE test methods for identifying nonsevere ocular irritants and substances not labeled as irritants. The report also includes recommendations on the Cytosensor® Microphysiometer (CM) test method, which was not part of the 2006 evaluation. The report summarizes the validation status of each test method and provides the ICCVAM-recommended BCOP, CM, HET-CAM, ICE, and IRE test method protocols.

---

[1] Available at: http://www.preventblindness.org/resources/factsheets/Eye_Injuries_FS93.PDF
[2] Available at: http://www.geteyesmart.org/eyesmart/injuries/home.cfm
[3] From the CPSC NEISS Database, 2007
[4] Available at: http://www.cdc.gov/niosh/topics/eye/
[5] Test Guideline 437. Bovine corneal opacity and permeability test method for identifying ocular corrosives and severe irritants; Test Guideline 438. Isolated chicken eye test method for identifying ocular corrosives and severe irritants. Both In: OECD Guidelines for Testing of Chemicals. Paris:Organisation for Economic Co-operation and Development

As part of ICCVAM's ongoing international collaborations, scientists from the European Centre for the Validation of Alternative Methods (ECVAM) and the Japanese Center for the Validation of Alternative Methods (JaCVAM) served as liaisons to the ICCVAM Ocular Toxicity Working Group (OTWG). ICCVAM, NICEATM, and the OTWG prepared (1) draft background review documents (BRDs) describing the validation status of each test method, including reliability and accuracy, and (2) draft test method recommendations for their usefulness and limitations.

ICCVAM released these documents to the public for comment prior to a meeting of an independent international scientific peer review panel (Panel). The Panel met in public session on May 19–21, 2009, and prepared a report summarizing its conclusions and recommendations. The Panel report was provided to the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) along with the draft BRDs, draft test method recommendations, and all public comments. A detailed timeline of the evaluation is included with this report.

ICCVAM solicited and considered public comments and stakeholder involvement throughout the test method evaluation process. ICCVAM considered the SACATM comments, the conclusions of the Panel, and all public comments before finalizing the ICCVAM test method recommendations for each test method. The recommendations and the BRDs, which are provided as appendices, are incorporated in this ICCVAM test method evaluation report. As required by the ICCVAM Authorization Act, ICCVAM will forward its recommendations to U.S. Federal agencies for consideration. Federal agencies must respond to ICCVAM within 180 days after receiving the ICCVAM test method recommendations. ICCVAM recommendations are available to the public on the NICEATM–ICCVAM website, and agency responses will also be made available on the website as they are received.

We gratefully acknowledge the many individuals who contributed to the preparation, review, and revision of this report. We especially recognize the Panel members for their thoughtful evaluations and generous contributions of time and effort. Special thanks are extended to Dr. A. Wallace Hayes for serving as the Panel Chair and to Dr. Paul Bailey, Dr. Donald Sawyer, Dr. Kirk Tarlo, and Dr. Daniel Wilson for their service as Evaluation Group Chairs. We thank the OTWG for assuring a meaningful and comprehensive review. We especially thank Dr. Jill Merrill (U.S. Food and Drug Administration Center for Drug Evaluation and Research) and Dr. Karen Hamernik (EPA, until April 2009) for serving as Co-Chairs of the OTWG. Integrated Laboratory Systems, Inc., the NICEATM support contractor, provided excellent scientific support, for which we thank Dr. David Allen, Dr. Jonathan Hamm, Nelson Johnson, Dr. Brett Jones, Dr. Elizabeth Lipscomb, and James Truax. Finally, we thank the ECVAM liaisons Drs. João Barroso, Thomas Cole, and Valerie Zuang and the JaCVAM liaison Dr. Hajime Kojima for their participation and contributions.

Marilyn Wind, Ph.D.
Deputy Associate Executive Director
Directorate for Health Sciences
U.S. Consumer Product Safety Commission
Chair, ICCVAM

William S. Stokes, D.V.M., DACLAM
Rear Admiral/Assistant Surgeon General, U.S. Public Health Service
Director, NICEATM
Executive Director, ICCVAM

Executive Summary

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) recently evaluated the validation status of test methods to identify substances that cause reversible eye injuries or do not cause sufficient eye damage to require hazard labeling: the bovine corneal opacity and permeability (BCOP), Cytosensor® Microphysiometer (CM), hen's egg test–chorioallantoic membrane (HET-CAM), isolated chicken eye (ICE), and isolated rabbit eye (IRE) test methods. Nearly all ocular safety testing has been conducted using the *in vivo* Draize rabbit eye test (Draize et al. 1944) to evaluate the potential for substances to cause ocular irritation and other ocular injuries, an acute reaction that may involve corneal cloudiness and ulceration, swelling and redness of the conjunctiva, and/or visible damage to the inside of the eye (iritis). The BCOP, CM, HET-CAM, ICE, and IRE methods are *in vitro* test methods that predict the extent of ocular damage that might occur *in vivo* without requiring the use of live animals. This test method evaluation report provides ICCVAM's recommendations for each *in vitro* test method as an alternative to the Draize rabbit eye test, based on demonstrated validity (usefulness and limitations). This report includes (1) protocols recommended by ICCVAM for future data collection and evaluation for the BCOP, CM, HET-CAM, ICE, and IRE test methods, (2) final background review documents (BRDs) describing the validation status of these test methods, and (3) recommendations for future studies.

Following a nomination by the U.S. Environmental Protection Agency (EPA) requesting an evaluation of several alternative methods and approaches for reducing, replacing, and refining the use of rabbits in the current *in vivo* eye irritation test method, the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), ICCVAM, and the ICCVAM Ocular Toxicology Working Group prepared draft BRDs and draft test method recommendations. The drafts were provided to an independent international scientific peer review panel (hereafter "Panel") and to the public for comment. The Panel met in public session on May 19-21, 2009, to discuss its peer review of the ICCVAM draft BRDs and to provide conclusions and recommendations regarding the validation status of the BCOP, CM, HET-CAM, ICE, and IRE test methods. The Panel also reviewed how well the information contained in the draft BRDs supported ICCVAM's draft test method recommendations.

In finalizing this test method evaluation report and the BRDs, which are included here as appendices, ICCVAM considered (1) the conclusions and recommendations of the Panel, (2) comments from ICCVAM's Scientific Advisory Committee on Alternative Toxicological Methods (SACATM), and (3) public comments.

The Bovine Corneal Opacity and Permeability (BCOP) Test Method

ICCVAM Recommendations: BCOP Test Method Usefulness and Limitations
ICCVAM concludes that the accuracy and reliability of the BCOP test method does **not** support its use as a screening test to distinguish substances not labeled as irritants (EPA Category IV, European Union [EU] Not Labeled, Federal Hazardous Substances Act [FHSA] Not Labeled, United Nations Globally Harmonized System of Classification and Labelling of Chemicals [GHS] Not Classified) from all other hazard categories (EPA Category I, II, or III; EU R41 or R36; FHSA Irritant; GHS Category 1, 2A, or 2B) when results are to be used specifically to classify and label substances under the EPA, EU, FHSA, or GHS classification systems. For the BCOP validation database of 211 substances, false positive rates were high, ranging from 53% (24/45) to 70% (63/90), depending on the hazard classification system used. Therefore, all positive results from these tests would require additional testing in a valid test system that can accurately characterize whether such substances require hazard labeling. False negative rates were 0% for the EU (0/54) and GHS (0/97) classification systems, 5% (6/132) for the FHSA classification system, and 6% (8/142) for the EPA classification system.

Among the eight EPA false negatives were three substances (3/8 [38%]) that were classified as EPA eye irritants based on at least one rabbit with corneal injuries and opacity that did not resolve until day 3 of the study. A fourth substance was classified as an EPA eye irritant based on all six rabbits with a conjunctival redness score of 2 (n = 4; *diffuse, crimson color of the conjunctiva, individual blood vessels not easily discernable*) or 3 (n = 2; *diffuse beefy red*). The conjunctival redness scores for two of these animals did not recover to a score of 1 (*some blood vessels definitely hyperemic*) until day 6 of the study. The conjunctival redness scores for the remaining four rabbits recovered to a score of 1 on day 2 of the study. These four EPA false negative substances were also false negatives for the FHSA classification system. Given the significant lesions associated with these false negative substances, the BCOP test method cannot be recommended as a screening test to identify substances not labeled as irritants (i.e., EPA Category IV, FHSA Not Labeled) for the EPA or FHSA classification systems.

Furthermore, although the false negative rate was 0% (0/97) for the GHS classification scheme, the GHS does not classify substances as eye hazards that produce the corneal and conjunctival injuries described above, which are required to be labeled as eye hazards according to the EPA and FHSA classification systems. These findings led NICEATM-ICCVAM to look more closely at the GHS eye hazard classification criteria. NICEATM evaluated results from rabbit eye test studies from two independent databases: (1) 149 studies obtained from a publicly available database (ECETOC 1998) and (2) 144 studies included in the Organization for Economic Cooperation and Development (OECD) Detailed Review Document on Classification Systems for Eye Irritation/Corrosion in OECD Member Countries (OECD 1999). These data, which are included here as an appendix, confirmed that approximately 30% of the substances requiring labeling for eye irritation hazard based on current U.S. hazard classification requirements (EPA and FHSA) are not labeled as eye irritation hazards by the GHS system. This includes at least 70% of currently labeled EPA Category III irritants (those causing eye injuries persisting for 24 hours to 7 days) that would not require hazard labeling using the GHS system. The nature, severity, and duration of these eye injuries suggest the potential to cause human injury. The purpose of ocular toxicity labeling is to communicate potential hazards of chemicals and products to workers and consumers so that appropriate measures can be taken to avoid accidental or inadvertent contact with the eye. In addition, ocular safety labels provide the necessary first aid measures that should be taken in the event of accidental exposures.

The GHS was established based on principles agreed to by participants, which included assuring that "the level of protection offered to workers, consumers, the general public and the environment should not be reduced as a result of harmonizing the classification and labeling systems" (UN 2007). ICCVAM has conducted technical analyses to support the development of appropriate recommendations for GHS options that would continue to provide protection that is at least equivalent to current U.S. eye irritation hazard classification and labeling requirements. ICCVAM recommends that U.S. agencies consider the GHS eye irritation hazard classification criteria and hazard categories and the level of protection they provide compared to current U.S. hazard classification systems.

Federal law requires agencies to determine that new test methods recommended by ICCVAM generate data that are at least equivalent to data generated by current test methods required or recommended by each agency for hazard identification purposes. Until the issues associated with the GHS system as outlined above are further discussed, ICCVAM is deferring final recommendations on the usefulness and limitations of using the BCOP test method as a screening test to identify substances not labeled as irritants according to the GHS classification system.

ICCVAM Recommendations: BCOP Test Method Protocol

For use of the BCOP test method as a screening test to identify substances as ocular corrosives and severe irritants (EPA Category I, EU R41, GHS Category 1), ICCVAM recommends using the

updated ICCVAM BCOP test method protocol included as an appendix to this report. All future studies intended to further characterize the usefulness and limitations of the BCOP test method should be conducted using this protocol.

ICCVAM Recommendations: BCOP Future Studies
ICCVAM recommends additional studies to further characterize and potentially improve the usefulness and applicability of the BCOP test method to distinguish ocular irritants from all hazard categories:

- Additional optimization studies/evaluations should be conducted to improve the correct classification of mild and moderate ocular irritants and substances not labeled as irritants. After optimization, additional studies to further assess the reliability and accuracy of the test method are recommended.
- Histopathological evaluation of the corneal tissue, using standardized procedures, should be included when the BCOP test method is used. Such data will help develop decision criteria and future assessments on the usefulness of this endpoint for classifying and labeling substances, especially those that may otherwise produce borderline or false negative results.
- Users of the BCOP test method should provide all data that are generated from future studies, because they could help to further characterize the usefulness and limitations of the BCOP test method to identify all ocular hazard categories.

ICCVAM Recommendations: BCOP Performance Standards
Based on the available data and associated performance described above, ICCVAM recommends that the development of performance standards for the BCOP test method is not warranted at this time.

Validation Status of the BCOP Test Method
The BCOP test method is an *in vitro* method that provides short-term maintenance of physiological and biochemical function of the bovine cornea. Quantitative changes in opacity and fluorescein permeability are assessed as indicators of potential ocular irritation.

The accuracy of the BCOP test method was compared to hazard categories based on *in vivo* Draize rabbit eye test data according to the EPA, EU, FHSA, or GHS systems using the current BCOP validation database of 211 substances. When the BCOP test method was used to distinguish substances not labeled as irritants (EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified) from all other categories, accuracy ranged from 64% (76/118) to 83% (161/194), depending on the hazard classification system used. While false positive rates were high (53% [24/45] to 70% [63/90], depending on the hazard classification system used), the false negative rates were low (5% [6/132] for the FHSA system, 6% [8/141] for EPA the system, and 0% [0/54 or 0/97] for the EU and GHS systems, respectively).

Qualitative analyses of interlaboratory reproducibility were conducted to evaluate how well the BCOP hazard classifications agreed among the participating laboratories from the three different interlaboratory validation studies (Balls et al. 1995; Gautheron et al. 1994; and Southee 1998). These evaluations were based on the use of the BCOP test method (1) to identify all ocular hazard categories according to the EPA, EU, or GHS systems, and (2) to distinguish substances not labeled as irritants from all other ocular hazard categories. For both approaches, there was 100% agreement among the multiple laboratories in each study for a majority of the correctly identified ocular irritant hazard categories. Because the performance of the BCOP test method was similar for the EPA and FHSA hazard classification systems, additional reliability analyses were not conducted for the FHSA hazard classification system.

The Cytosensor Microphysiometer (CM) Test Method

ICCVAM Recommendations: CM Test Method Usefulness and Limitations
ICCVAM concludes that the accuracy and reliability of the CM test method support its use as a screening test to identify water-soluble substances (water-soluble surfactants, surfactant-containing formulations, and nonsurfactants) as ocular corrosives and severe irritants (EPA Category I, EU R41, GHS Category 1) in a tiered-testing strategy, as part of a weight-of-evidence approach. False positive rates ranged from 0% (0/17 or 0/18) to 10% (3/29), and false negative rates ranged from 9% (2/23) to 50% (6/12), depending on the classification system used and the type of substance tested. A substance that tests negative with the CM test method would need to be tested in another test method that can identify possible *in vitro* false negative ocular corrosives and severe irritants and distinguish between moderate and mild ocular irritants. Currently, the Draize rabbit eye test is the only test method that can make such a distinction.

ICCVAM further concludes that the accuracy and reliability of the CM test method are sufficient to support its use as a screening test to distinguish water-soluble surfactant chemicals and certain types of surfactant-containing formulations (e.g., cosmetics and personal care product formulations, but not pesticide formulations) as substances not labeled as irritants (EPA Category IV, EU Not Labeled, FHSA Not Labeled) from all other hazard categories (EPA Category I, II, III; EU R41, R36; FHSA Irritant) when results are to be used specifically to classify and label substances under the EPA, EU, and FHSA classification systems. As noted above, until the issues associated with the GHS classification system are further discussed (see "BCOP Test Method Usefulness and Limitations"), ICCVAM is deferring final recommendations on the usefulness and limitations of using the CM test method as a screening test to identify substances not labeled as irritants according to the GHS classification system.

When the CM test method was used to distinguish substances not listed as irritants from all other hazard categories the validation database of 53 water-soluble surfactants and surfactant-containing formulations, false positive rates were high, ranging from 50% (3/6) to 69% (18/26), depending on the hazard classification system used. However, such positive results would require additional testing in a valid test system that can accurately characterize whether such substances require hazard labeling. Positive results would also need to be additionally tested with methods that can correctly identify moderate and mild ocular irritants. False negative rates ranged from 0% (0/27, 0/28, or 0/40) to 2% (1/42 or 1/47) compared to results from the Draize rabbit eye test. The one false negative substance was EPA Category III or FHSA Irritant based on *in vivo* data. For this substance, six test animals were included in the *in vivo* test. One test animal had no observable effects, three test animals had conjunctival redness (score = 1), and two test animals had corneal opacity (score = 1) that cleared after one day.

Because of the high false negative rates (24% [5/21] to 40% [8/20]for the CM test method when testing water-soluble nonsurfactant substances and formulations, the CM test method is **not** recommended as a screening test to identify substances not labeled as irritants among these types of substances.

Given that the CM test method (INVITTOX Protocol 102) is proposed for use as a screening test to identify ocular corrosives and severe irritants and substances not labeled as irritants, users may want to consider using the CM test method before using another *in vitro* ocular test method for testing these types of substances. However, water-soluble substances that are not identified as ocular corrosives and severe irritants or water-soluble surfactant chemicals and specific types of surfactant-containing formulations that are not identified as substances not labeled as irritants with the CM test method would need to be tested in another test method able to correctly classify substances into each of the four EPA or GHS hazard classification categories. Currently, the only test method accepted for these purposes is the Draize rabbit eye test. Because the CM test method has a high false positive rate

for substances not labeled as irritants (50% [3/6] to 69% [18/26], depending on the hazard classification system used), users may not want to use it if the intended use is to start with identifying substances not labeled as irritants.

ICCVAM Recommendations: CM Test Method Protocol
For use of the CM test method as a screening test to identify water-soluble substances as ocular corrosives and severe irritants (EPA Category I, EU R41, GHS Category 1) or to identify substances not labeled as irritants (EPA Category IV, EU Not Labeled, FHSA Not Labeled), ICCVAM recommends using the updated ICCVAM CM INVITTOX Protocol 102[6] that is included as an appendix to this report. All future studies intended to further characterize the usefulness and limitations of the CM test method should be conducted using this protocol.

ICCVAM Recommendations: CM Future Studies
ICCVAM recommends that additional studies be conducted to further characterize the usefulness and limitations of the CM test method for use as a screening test to identify ocular corrosives and severe irritants (EPA Category I, GHS Category 1, EU R41) and substances not labeled as irritants (EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified). This includes additional testing using a broader range of materials to expand the recommended types of substances appropriate for testing.

ICCVAM recommends that a subset of the ICCVAM-recommended reference substances for validation of *in vitro* ocular toxicity test methods for the evaluation of ocular corrosives and severe irritants[7] be tested in the CM test method in order to provide for more direct assessment of the CM test method's utility as a screening test for identifying ocular corrosives and severe irritants. Similarly, a reference set could also be selected from this list for the purposes of assessing the utility of the CM test method as a screening test for identifying substances not labeled as irritants.

Finally, ICCVAM recommends future optimization studies to increase the ability of the CM test method to identify all categories of ocular irritancy hazard classification according to the EPA, EU, or GHS hazard classification systems. This will require more substances in the moderate and mild ocular irritant categories (EPA Category II and III, EU Category R36, or GHS Category 2A and 2B, respectively) be identified and tested.

ICCVAM Recommendations: CM Performance Standards
Based on the available data and associated performance described above, ICCVAM recommends that the development of performance standards for the CM test method is not warranted at this time.

Validation Status of the CM Test Method
The CM test method exposes a population of cells to increasing concentrations of a test substance. The concentration that leads to a 50% decline in the metabolic rate of the cells (the MRD_{50}) is used as an indicator of ocular irritancy potential. An abbreviated version of the European Centre for the Validation of Alternative Methods (ECVAM) CM BRD that does not include confidential business information describes the current validation status of the CM test method, including what is known about its reliability and accuracy, the scope of substances tested, and standardized protocols for the validation study. The following is a synopsis of the information contained within three peer-reviewed publications (Balls et al. 1995; Gettings et al. 1996; Brantom et al. 1997) described in the ECVAM CM BRD and used in the ICCVAM review.

---

[6] Available at http://ecvam-dbalm.jrc.ec.europa.eu/
[7] http://iccvam.niehs.nih.gov/methods/ocutox/ivocutox/ocu_tmer.htm

The database of 53 water-soluble surfactants tested in the CM test method included 21 surfactant chemicals and 32 surfactant-containing formulations tested across seven different laboratories. Using INVITTOX Protocol 102 to identify ocular corrosives and severe irritants among the water-soluble surfactants and surfactant-containing formulations, the false positive rate ranged from 3% (1/30) to 10% (3/29), depending on the hazard classification system used, compared to *in vivo* results. The three false positives when using the EPA classification system are classified as Category II (n = 2) or III (n = 1) based on *in vivo* data. The one false positive when using the GHS and EU classification systems is classified as Not Classified and Not Labeled, respectively, based on *in vivo* data. The false negative rate ranged from 9% (2/23) to 22% (5/23), depending on the hazard classification system used, compared to *in vivo* results. In each case, these substances were classified as moderate or mild irritants *in vitro* based on the EPA, EU, and GHS classification systems (i.e., EPA Category II or III; EU R36; or GHS Category 2A or 2B).

The nonsurfactant substances database (n = 29) consisted of 27 water-soluble nonsurfactant chemicals, which included a range of chemical classes (e.g., acids, alcohols, alkalis, and ketones), and water-soluble nonsurfactant formulations (n = 2) tested in seven laboratories. Using INVITTOX Protocol 102 to identify ocular corrosives and severe irritants among the nonsurfactant substances, the false positive rate was 0% (0/17 or 0/18) for all hazard classification systems compared to *in vivo* results. The false negative rate ranged from 29% (2/7) to 50% (6/12), depending on the hazard classification system used, compared to *in vivo* results. Two substances were false negatives when using the EPA classification system and were classified *in vitro* as either Category II/III (n = 1) or IV (n = 1). Five substances were false negatives when using the GHS classification system and were classified *in vitro* as either Category 2A/2B (n = 4) or Not Labeled (n = 1). Six substances were false negatives when using the EU classification system and were classified *in vitro* as either R36 (n = 5) or Not Labeled (n = 1).

Using INVITTOX Protocol 102 to identify substances not labeled as irritants among the database of 53 water-soluble surfactants and surfactant-containing formulations, the false negative rate ranged from 0% (0/27 or 0/28, or 0/40) to 2% (1/46 or 1/47), depending on the hazard classification system used, compared to *in vivo* results. The one substance that was a false negative is classified as EPA Category III based on *in vivo* data from a six-rabbit *in vivo* test. One rabbit had no observable effects, three rabbits had conjunctival redness (score = 1), and two rabbits had corneal opacity (score = 1) that cleared after one day. The false positive rate ranged from 50% (3/6) to 69% (18/ 26), depending on the hazard classification system used, compared to *in vivo* results. Three substances were false positives when using the EPA and FHSA classification systems and were classified *in vitro* as Category II/III or Irritant, respectively. Seventeen substances were false positives when using the GHS classification system and were classified *in vitro* as Category 2A/2B (n = 16) or Category 1 (n = 1). Eighteen substances were false positives when using the EU classification system and were classified *in vitro* as R36 (n = 17) or R41 (n = 1).

Using INVITTOX Protocol 102 to identify substances not labeled as irritants among the database of 29 nonsurfactant substances, the false negative rate ranged from 24% (5/21) to 40% (8/20), and the false positive rate ranged from 25% (1/4 or 2/8) to 40% (2/5), depending on the hazard classification system used, compared to *in vivo* results.

Intralaboratory reproducibility was assessed based on calculated coefficients of variation (CVs) for MRD_{50} values for two different studies. Mean CVs ranged from 10% to 24% and tended to be slightly higher for surfactant substances than for nonsurfactant substances.

Interlaboratory reproducibility of the CM test method was also assessed using the data from validation studies by the European Commission/Home Office (EC/HO; Balls et al. 1995) and European Cosmetic, Toiletry and Perfumery Association (COLIPA; Brantom et al. 1997), which included four laboratories and two laboratories, respectively. Mean CVs in the EC/HO study ranged

from 16% to 37% for surfactant substances and up to 51% for nonsurfactant substances. For surfactant materials, all four laboratories using the CM test method had 100% agreement for 55% (6/11) of the test substances; 75% of the laboratories had identical results for 27% (3/11) of the test substances; and 50% of the laboratories had agreement for 18% (2/11) of the test substances. For nonsurfactant substances, agreement among the laboratories was 100% for 48% (11/23) of the test substances, 75% for 22% (5/23) of the test substances, 67% for 4% (1/23) of the test substances, and 50% for 13% (3/23) of the test substances.

For the COLIPA study, substances were divided into surfactant materials, surfactant-based formulations and mixtures, and nonsurfactant substances. Two laboratories had mean between-laboratory CVs ranging from 16% to 23% for surfactant materials, approximately 16% for surfactant-based formulations and mixtures, and 32% to 51% for nonsurfactant substances. For surfactant materials, the laboratories had 100% agreement for 90% (9/10) of the test substances and 0% agreement for 10% (1/10) of the test substances. For surfactant-based formulations and mixtures, the laboratories had 100% agreement for 100% (7/7) of the test substances. For nonsurfactant substances, the laboratories had 100% agreement for 78% (7/9) of the test substances and 0% agreement for 22% (2/9) of the test substances.

The Hen's Egg Test – Chorioallantoic Membrane (HET-CAM) Test Method

ICCVAM Recommendations: HET-CAM Test Method Usefulness and Limitations
ICCVAM concludes that the accuracy and reliability of the HET-CAM test method does **not** support its use as a screening test to distinguish substances not labeled as irritants (EPA Category IV, EU Not Labeled, FHSA Not Labeled) from all other hazard categories (EPA Category I, II, or III; EU R41 or R36; FHSA Irritant) when results are to be used specifically to classify and label substances under the EPA, EU, or FHSA classification systems.

The available validation database for the HET-CAM test method has remained unchanged since the original ICCVAM evaluation (ICCVAM 2006b). For the HET-CAM validation database of 60 surfactants and oil/water emulsions, false positive rates were 60% (9/15) to 69% (22/32) and false negative rates were 0% (0/26) to 9% (4/45). Among the four false negatives, 100% (4/4) were EPA Category III substances based on conjunctival redness scores of 2 that required at least three days to resolve. For one of the substances, one of the six rabbits tested had a conjunctival redness score of 2 that required 14 days to resolve. Four of the remaining five rabbits in this study had conjunctival redness scores of 2 that resolved within three days; the last rabbit did not have this lesion. However, there were too few substances in the moderate irritant categories to have sufficient confidence in the ability of HET-CAM to distinguish them from the substances not labeled as irritants category (there were only 2 EPA Category II substances).

ICCVAM Recommendations: HET-CAM Test Method Protocol
The updated ICCVAM-recommended HET-CAM test method protocol is included as an appendix to this report. The protocol has been modified from a generic description of the Irritation Score (IS) analysis method to include a more detailed IS(A) analysis method to be used for prospective studies. However, a description of the IS(B) method is included for retrospective analyses, where IS(B) analysis method data could be converted to fixed time points similar to those used for the IS(A) analysis method. All future studies intended to further characterize the usefulness and limitations of the HET-CAM test method should be conducted using this protocol.

ICCVAM Recommendations: HET-CAM Future Studies
ICCVAM recommends additional studies to further characterize and potentially improve the usefulness and applicability of the HET-CAM test method to distinguish ocular irritants from all hazard categories:

- Additional studies should be conducted to further optimize the HET-CAM test method decision criteria that would be used to identify ocular corrosives and severe irritants (EPA Category I, EU R41, GHS Category 1), as well as moderate irritants (EPA Category II, EU R36, GHS Category 2A) and mild irritants (EPA Category III, GHS Category 2B), as defined by the EPA, GHS, or EU classification systems. Such studies could potentially improve the usefulness of the HET-CAM test method for identifying these types of substances.
- The types of substances appropriate for testing should be expanded to include a broader range of chemical and product classes.
- Users of the HET-CAM test method should provide all data that are generated from future studies, because they could help to further characterize the usefulness and limitations of the HET-CAM test method to identify all ocular hazard categories.

ICCVAM Recommendations: HET-CAM Performance Standards
Based on the available data and associated performance described above, ICCVAM recommends that the development of performance standards for the HET-CAM test method is not warranted at this time.

Validation Status of the HET-CAM Test Method
ICCVAM reviewed HET-CAM performance compared to the Draize rabbit eye test for each classification system (EPA, EU, and GHS) using each of the six HET-CAM protocols (IS[A], IS[B], Q-Score, S-Score, IS, and ITC protocols). With the exception of the IS(A) and IS(B) protocols, all protocols classified at least one *in vivo* moderate or severe irritant substance as a substance not labeled as an irritant (EPA Category IV, EU Not Labeled, GHS Not Classified). The IS(B) overpredicted more than 90% (39/42) of the GHS Not Classified substances. Therefore, more extensive analyses of HET-CAM were restricted to the IS(A) protocol.

No new HET-CAM data have been obtained since the ICCVAM evaluation of the HET-CAM test method for identifying ocular corrosives and severe irritants (ICCVAM 2006b). Overall accuracy in distinguishing substances not labeled as irritants (EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified) from all other categories ranged from 62% (36/58) to 80% (44/55), depending on the hazard classification system used. False positive rates were 60% (9/15) to 69% (22/32) and false negative rates were 0% (0/26) to 9% (4/45). Among the four false negatives, 100% (4/4, all oil/water emulsion cosmetic formulations) were EPA Category III substances based on conjunctival redness scores of 2 that required at least three days to resolve. For one of the substances, one out of the six rabbits tested had a conjunctival redness score of 2 that required 14 days to resolve. Four of the remaining five rabbits in this study had conjunctival redness scores of 2 that resolved within three days; the last rabbit did not have this lesion.

Quantitative and qualitative evaluations of HET-CAM test method reliability have been conducted previously (ICCVAM 2006b). Because the database used for the current evaluation of the HET-CAM test method has not changed, the quantitative evaluation of test method reliability remains unchanged. Additional qualitative analyses of interlaboratory reproducibility were conducted to evaluate how well the HET-CAM hazard classifications agreed among the five laboratories that participated in the interlaboratory validation study (Hagino et al. 1999). These evaluations were based on the use of the HET-CAM test method (1) to identify all ocular hazard categories according to the EPA, EU, or GHS systems, and (2) to distinguish substances not labeled as irritants from all other ocular hazard

categories. For both approaches, there was 100% agreement among the multiple laboratories in each study for a majority of the correctly identified ocular irritant hazard categories. Because the performance of the HET-CAM test method was similar for the EPA and FHSA hazard classification systems, additional reliability analyses were not conducted for the FHSA hazard classification system.

The Isolated Chicken Eye (ICE) Test Method

ICCVAM Recommendations: ICE Test Method Usefulness and Limitations
ICCVAM concludes that the accuracy and reliability of the ICE test method does **not** support its use as a screening test to distinguish substances not labeled as irritants (EPA Category IV, EU Not Labeled, FHSA Not Labeled) from all other hazard categories (EPA Category I, II, or III; EU R41 or R36; FHSA Irritant) when results are to be used specifically to classify and label substances under the EPA, EU, or FHSA classification systems.

The available validation database for the ICE test method has remained unchanged since the original ICCVAM evaluation (ICCVAM 2006c). For the ICE validation database of 175 substances, false positive rates were 11% (10/93) to 34% (27/79) and false negatives rates were 6% (4/62) to 22% (13/60). Among the false negatives, at least one substance was classified as an ocular corrosive/severe irritant based on Draize rabbit eye test data (n = 1 each for the EPA and GHS systems, and n = 6 for the EU system). Considering the public health impact of misclassifying a corrosive substance as Not Labeled, these false negative results cannot be minimized.

ICCVAM Recommendations: ICE Test Method Protocol
For use of the ICE test method as a screening test to identify substances as ocular corrosives and severe irritants (EPA Category I, GHS Category 1, EU R41), ICCVAM recommends using the updated ICCVAM ICE test method protocol that is included as an appendix to this report. All future studies intended to further characterize the usefulness and limitations of the ICE test method should be conducted using this protocol.

ICCVAM Recommendations: ICE Future Studies
ICCVAM recommends additional studies to further characterize and potentially improve the usefulness and applicability of the ICE test method to distinguish ocular irritants from all hazard categories:

- Additional optimization studies should be conducted to improve the correct classification of mild and moderate ocular irritants and substances not labeled as irritants. After optimization, additional studies to further assess the reliability and accuracy of the test method are recommended.
- Histopathological evaluation of the corneal tissue, using standardized procedures, should be included when the ICE test method is used. Such data will help develop decision criteria and future assessments on the usefulness of this endpoint for classifying and labeling substances, especially those that may otherwise produce borderline or false negative results.
- Users of the ICE test method should provide all data that are generated from future studies, because they could help to further characterize the usefulness and limitations of the ICE test method to identify all ocular hazard categories.

ICCVAM Recommendations: ICE Performance Standards
Based on the available data and associated performance described above, ICCVAM recommends that the development of performance standards for the ICE test method is not warranted at this time.

Validation Status of the ICE Test Method
No new ICE data have been obtained since the ICCVAM evaluation of the ICE test method for identifying ocular corrosives and severe irritants (ICCVAM 2006c). Overall accuracy in distinguishing substances not labeled as irritants (EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified) from all other categories ranged from 78% (110/141) to 85% (130/153), depending on the hazard classification system used. False positive rates were 11% (10/93) to 34% (27/79) and false negative rates were 6% (4/62) to 22% (13/60). Among these false negatives, at least one substance was classified as an ocular corrosive/severe irritant based on Draize rabbit eye test data (n = 1 each for the EPA and GHS systems, and n = 6 for the EU system). Considering the public health impact of misclassifying a corrosive substance as Not Labeled, these false negative results cannot be minimized.

Quantitative and qualitative evaluations of ICE test method reliability have been conducted previously (ICCVAM 2006c). Because the database used for the current evaluation of the ICE test method has not changed, the quantitative evaluation of test method reliability remains unchanged. Additional qualitative analyses of interlaboratory reproducibility were conducted to evaluate how well the ICE hazard classifications agreed among the four laboratories that participated in the interlaboratory validation study (Balls et al. 1995). These evaluations were based on the use of the ICE test method (1) to identify all ocular hazard categories according to the EPA, EU, or GHS systems, and (2) to distinguish substances not labeled as irritants from all other ocular hazard categories. For both approaches, there was 100% agreement among the multiple laboratories in each study for a majority of the correctly identified ocular irritant hazard categories. Because the performance of the ICE test method was similar for the EPA and FHSA classification systems, additional reliability analyses were not conducted for the FHSA classification system.

The Isolated Rabbit Eye (IRE) Test Method

ICCVAM Recommendations: IRE Test Method Usefulness and Limitations
The available validation database for the IRE test method has remained unchanged since the original ICCVAM evaluation (ICCVAM 2006d). Because of the lack of a standardized protocol and insufficient data using all four recommended IRE endpoints, ICCVAM concludes that additional studies are needed before definitive recommendations on the accuracy and reliability of the IRE test method can be made.

ICCVAM Recommendations: IRE Test Method Protocol
An ICCVAM-recommended test method protocol for the IRE test method that should be used for all future IRE studies is included as an appendix to this report. The recommended protocol remains unchanged from the previous ICCVAM evaluation (ICCVAM 2006e) and includes four endpoints that should be measured: maximal corneal opacity (opacity x area), maximal corneal swelling, fluorescein penetration (intensity x area), and assessment of epithelial integrity (at 0.5, 1, 2, 3, and 4 hours after test substance administration.

ICCVAM Recommendations: IRE Future Studies
ICCVAM recommends additional studies to further characterize and potentially improve the usefulness and applicability of the IRE test method to distinguish ocular irritants from all other hazard categories:

- Additional evaluation studies should be conducted to increase the current IRE database and optimize the IRE test method decision criteria. Once these studies are conducted, ICCVAM recommends that additional validation studies be conducted to further evaluate the relevance and reliability of the IRE test method.
- Histopathological evaluation of the corneal tissue, using standardized procedures, should be included when the IRE test method is used. Such data will help develop decision

criteria and future assessments on the usefulness of this endpoint for classifying and labeling substances, especially those that may otherwise produce borderline or false negative results.

- Users of the IRE test method should provide all data that are generated from future studies, because they could help to further characterize the usefulness and limitations of the IRE test method to identify all ocular hazard categories.

ICCVAM Recommendations: IRE Performance Standards
Based on the available data described above, ICCVAM recommends that the development of performance standards for the IRE test method is not warranted at this time.

Validation Status of the IRE Test Method
The performance section of the IRE BRD (ICCVAM 2006d) uses data from Balls et al. (1995), Gettings et al. (1996), and Guerriero et al. (2004). These references were examined for decision criteria that would help classify moderate and mild irritants. There are insufficient data using all four recommended IRE endpoints (corneal opacity, fluorescein penetration, corneal swelling, and observations of significant effect on corneal epithelium) to assess the accuracy and reliability of the IRE test method when all of these endpoints are evaluated in a single study. Furthermore, among the studies that included each endpoint, decision criteria focused on distinguishing ocular corrosives and severe irritants from all other ocular hazard categories (moderate and mild irritants and substances not labeled as irritants) and did not specify decision criteria for each ocular hazard category. For these reasons, an adequate evaluation of the IRE test method for its ability to distinguish substances not labeled as irritants from all other ocular hazard categories is not feasible at this time.

Because of the lack of quantitative IRE test method data for replicate experiments within an individual laboratory, the intralaboratory repeatability and reproducibility of the IRE test method could not be evaluated. However, multilaboratory qualitative and quantitative IRE test data were available for a collaborative study by the Commission of European Communities (CEC 1991) involving three laboratories and a validation study conducted by Balls et al. (1995) involving four laboratories. In the CEC (1991) study, each substance tested was assigned a EU classification (R41, R36, or nonirritant [EU 2001]) based on Draize rabbit eye test results. However, due to the lack of individual rabbit Draize scores, a reliability assessment for the CEC (1991) study using the GHS (UN 2007) or EPA (EPA 2003) classification criteria was not possible. The Balls et al. (1995) data were used for an evaluation of the interlaboratory reproducibility of the IRE test method according to the GHS (UN 2007), EPA (EPA 2003), and EU (EU 2001) classification systems.

ICCVAM Consideration of Public and SACATM Comments

The ICCVAM evaluation process incorporates a high level of transparency. This process is designed to provide numerous opportunities for stakeholder involvement, including submitting written public comments and providing oral comments at ICCVAM independent peer review panel meetings and SACATM meetings. **Table 7-1** lists the nine different opportunities for public comments that were provided during the ICCVAM evaluation of the validation status of alternative ocular safety testing methods and approaches. A total of 37 public comments were received. Comments received in response to or related to the *Federal Register* notices are also available on the NICEATM-ICCVAM website.[8]

[8] Available at http://ntp-apps.niehs.nih.gov/iccvambp/searchPubCom.cfm

This page intentionally left blank

1.0 Introduction

In October 2003, the U.S. Environmental Protection Agency (EPA) submitted to the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) a nomination requesting evaluation of several activities related to reducing, refining, and replacing the use of rabbits in the current *in vivo* eye irritation test method (announced in *Federal Register* [FR] notice 69 FR 13859, March 24, 2004). In response to this nomination, ICCVAM evaluated the validation status of the bovine corneal opacity and permeability (BCOP), hen's egg test–chorioallantoic membrane (HET-CAM), isolated chicken eye (ICE), and isolated rabbit eye (IRE) test methods. ICCVAM evaluated the test methods' ability to identify ocular corrosives and severe (irreversible) irritants using the EPA, United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS), and European Union (EU) classification systems.

ICCVAM considered the BCOP and ICE test methods to have sufficient performance to substantiate their use for regulatory hazard classification for some types of substances. The IRE and HET-CAM test methods lacked sufficient performance and/or sufficient data to substantiate their use for regulatory hazard classification. ICCVAM subsequently recommended that the BCOP and ICE test methods should be used in a tiered-testing strategy as part of a weight-of-evidence approach, where positive substances can be classified as ocular corrosives or severe irritants without the need for animal testing.

In accordance with the ICCVAM Authorization Act of 2000 (Public Law 106-545), these recommendations were made available to the public and provided to U.S. Federal agencies for consideration in the *ICCVAM Test Method Evaluation Report – In Vitro Ocular Toxicity Test Methods for Identifying Severe Irritants and Corrosives* (NIH Publication No. 07-4517, available at http://iccvam.niehs.nih.gov/methods/ocutox/ivocutox/ocu_tmer.htm). The ICCVAM recommendations were accepted by U.S. Federal agencies, and the Organisation for Economic Co-operation and Development (OECD) adopted the BCOP and ICE test methods as OECD Test Guidelines 437 and 438, respectively (OECD 2009a, 2009b). When used in this manner, the BCOP and ICE test methods should reduce the number of animals needed for ocular safety testing and refine animal use by avoiding the pain and distress associated with testing severely irritating and corrosive substances.

Among these final recommendations was a charge to further evaluate the usefulness and limitations of the BCOP, HET-CAM, ICE, and IRE test methods for the identification of nonsevere ocular irritants (i.e., substances that induce reversible ocular damage) and substances not labeled as irritants. In addition, the Cytosensor® Microphysiometer (CM) test method was evaluated as proposed by the European Centre for the Validation of Alternative Methods (ECVAM) as a possible alternative test method for the identification of ocular corrosives and severe irritants and substances not labeled as irritants. For these current evaluations, ICCVAM used the EPA, EU, Federal Hazardous Substances Act (FHSA), and GHS classification systems (EPA 2003; EU 2001; FHSA 2005; UN 2007). The FHSA classification system, which is based on the testing guidelines and associated criteria included in 16 CFR 1500.42 (CPSC 2003), was not used in the original analyses (i.e., ability of the test methods to identify ocular corrosives and severe irritants) because the FHSA ocular hazard category that is assigned based on results from the Draize rabbit eye test (Draize et al. 1944) does not distinguish between ocular corrosives and severe irritants and less severe irritants. For this reason, an evaluation to identify ocular corrosives and severe irritants using the FHSA classification system is not possible.

The ICCVAM Ocular Toxicity Working Group (OTWG) was charged with working with the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) in reviewing these *in vitro* alternatives. Drs. João Barroso, Thomas Cole, and Valerie Zuang were ECVAM liaisons, and Dr. Hajime Kojima was the Japanese Center for the Validation of Alternative Methods (JaCVAM) liaison to the OTWG.

To facilitate peer review, the OTWG and NICEATM, which administers ICCVAM and provides scientific and operational support for ICCVAM activities, prepared comprehensive draft background review documents (BRDs) that provided information and data from validation studies and the scientific literature for the BCOP, HET-CAM, ICE, and IRE test methods. A redacted BRD (i.e., an abbreviated version that does not include confidential business information) for the CM test method was prepared by ECVAM and submitted to NICEATM–ICCVAM for review.

A June 7, 2007, *Federal Register* notice (72 FR 31582, available at http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR_E7_10966.pdf) requested data and information on these test methods. In addition, an April 4, 2008 *Federal Register* notice (73 FR 18535, available at http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR-E8-6969.pdf) requested nominations of individuals to serve on an independent international scientific peer review panel (Panel). These requests were also disseminated via the ICCVAM electronic mailing list and through direct requests to over 100 stakeholders. In response to these requests, 12 individuals or organizations nominated members to the Panel; however, no test method data were submitted (see **Section 7.0**).

The BRDs form the basis for the ICCVAM test method recommendations described herein. The ECVAM and JaCVAM liaisons to the OTWG provided input and contributed throughout the evaluation process. A detailed timeline of the evaluation is provided in **Appendix A**. The ICCVAM-recommended test method protocol and the BRD for each test method are provided in **Appendices B through F**.

On March 31, 2009, ICCVAM announced the availability of the ICCVAM draft documents and a public Panel meeting to review the validation status of the test methods (74 FR 14556[1]). The ICCVAM draft BRDs and draft test method recommendations were posted on the NICEATM–ICCVAM website. All of the information provided to the Panel and all public comments received before the Panel meeting were made available on the NICEATM–ICCVAM website.[2]

The Panel met in public session on May 19–21, 2009, to review the ICCVAM draft BRDs for completeness and accuracy. The Panel then evaluated (1) the extent to which the draft BRDs addressed established validation and acceptance criteria and (2) the extent to which the BRDs supported ICCVAM's draft test method recommendations. Interested stakeholders from the public were provided opportunities to comment at the Panel meeting. The Panel considered these comments as well as those submitted prior to the meeting before concluding their deliberations. On July 12, 2009, ICCVAM posted the final report of the Panel's recommendations[3] (**Appendix G**) on the NICEATM–ICCVAM website for public review and comment (announced in 74 FR 33444).

ICCVAM provided SACATM with the draft BRDs, the draft Panel report, and all public comments for discussion at their meeting on June 25–26, 2009, where public stakeholders were given another opportunity to comment.

After SACATM's meeting, ICCVAM and the OTWG considered the SACATM comments, the Panel report, and all public comments (**Appendix H**) before finalizing the ICCVAM test method evaluation report and the BRDs provided in this report. As required by the ICCVAM Authorization Act, ICCVAM will make this test method evaluation report and the accompanying final BRDs available to the public and to U.S. Federal agencies for consideration. The relevant U.S. Federal laws, regulations, guidelines, and recommendations for eye irritation/corrosion testing are summarized in **Appendix I**. Federal agencies must respond to ICCVAM within 180 days after receiving ICCVAM test method recommendations. Agency responses will be made available to the public on the NICEATM–ICCVAM website as they are received.

---

[1] http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/E9-7220.pdf
[2] http://iccvam.niehs.nih.gov/methods/ocutox/PeerPanel09.htm
[3] http://iccvam.niehs.nih.gov/docs/ocutox_docs/OcularPRPRept2009.pdf

2.0 The Bovine Corneal Opacity and Permeability Test Method

The BCOP test method is an *in vitro* eye irritation test method using isolated bovine corneas that are byproducts from processing plants. In the BCOP test method, changes in corneal opacity caused by chemical damage are determined by measuring decreases in light transmission through the cornea. Changes in permeability of the cornea resulting from chemical damage are determined by measuring increases in the quantity of sodium fluorescein dye that passes through all corneal cell layers. Both measurements are used to calculate an *in vitro* irritancy score (IVIS), which is used to predict the *in vivo* ocular irritation/corrosion potential of a test substance.

ICCVAM previously evaluated the validation status of the BCOP test method as an *in vitro* alternative to the Draize rabbit eye test (Draize et al. 1944) to identify ocular corrosives and severe irritants (i.e., those that induce irreversible ocular damage; EPA Category I, EU R41, GHS Category 1). ICCVAM determined that the reproducibility and accuracy were sufficient to support its use for this purpose for some types of substances (ICCVAM 2006e). U.S. agencies and international organizations (OECD 2009a) have adopted the BCOP test method for this purpose. In the current evaluation, ICCVAM evaluated the validation status of the BCOP test method as an *in vitro* alternative to the Draize rabbit eye test for identifying nonsevere ocular irritants (i.e., those that induce reversible ocular damage [EPA Category II and III, EU R36, GHS Category 2A and 2B]) and substances not labeled as irritants (i.e., EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified) according to the EPA, EU, FHSA, and GHS classification systems (EPA 2003; EU 2001; FHSA 2005; UN 2007).

2.1 ICCVAM Recommendations

2.1.1 ICCVAM Recommendations: Test Method Usefulness and Limitations

Evaluation as a Screening Test to Identify Substances Not Labeled as Irritants
ICCVAM concludes that the accuracy and reliability of the BCOP test method do **not** support its use as a screening test to distinguish substances not labeled as irritants (i.e., EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified) from all other hazard categories (i.e., EPA Category I, II, and III; EU R41 or R36; FHSA Irritant; GHS Category 1, 2A, or 2B) when results are to be used specifically for hazard classification and labeling purposes under the EPA, EU, FHSA, or GHS classification systems (EPA 2003; EU 2001; FHSA 2005; UN 2007). For the validation database of 211 substances, false positive rates were high, ranging from 53% (24/45) to 70% (63/90) depending on the hazard classification system used. Accordingly, all positive results from these tests would require additional testing in a valid test system that can accurately characterize whether such substances require hazard labeling.

False negative rates were 0% for the EU (0/54) and GHS (0/97) classification systems, 5% (6/132) for the FHSA classification system, and 6% (8/142) for the EPA classification system. Among the EPA false negatives were three substances (3/8 [38%]) classified as EPA eye irritants based on at least one rabbit with corneal injuries and opacity that did not resolve until day 3 of the study. A fourth substance was classified as an EPA eye irritant based on all six rabbits with a conjunctival redness score of 2 (n = 4: *diffuse, crimson color of the conjunctiva, individual blood vessels not easily discernable*) or 3 (n = 2: *diffuse beefy red*). The conjunctival redness scores for two of these animals did not recover to a score of 1 (*some blood vessels definitely hyperemic*) until day 6 of the study. The conjunctival redness scores for the remaining four rabbits recovered to a score of 1 on day 2 of the study. These four EPA false negative substances were also false negatives for the FHSA classification system. Given the significant lesions associated with these false negative substances, the BCOP test method cannot be recommended as a screening test to identify substances not labeled as irritants (i.e., EPA Category IV, FHSA Not Labeled) for the EPA or FHSA classification systems (EPA 2003; FHSA 2005).

Furthermore, although the false negative rate is 0% (0/97) for the GHS classification system (UN 2007), the GHS does not classify as eye hazards substances that produce the corneal and conjunctival injuries described above. Such substances must be labeled as eye hazards according to the EPA and FHSA classification systems. These findings led NICEATM–ICCVAM to look more closely at the GHS eye hazard classification criteria. NICEATM evaluated results from rabbit eye test studies from two independent databases: (1) 149 studies obtained from a publicly available database (ECETOC 1998) and (2) 144 studies included in the Detailed Review Document (DRD) on Classification Systems for Eye Irritation/Corrosion in OECD Member Countries (OECD 1999). These data (**Appendix J**) confirmed that approximately 30% of the substances that require labeling as eye irritation hazards according to current U.S. hazard classification and labeling requirements (EPA and FHSA) are not labeled as eye irritation hazards by the GHS system. This includes at least 70% of currently labeled EPA Category III irritants (those causing eye injuries persisting for 24 hours to 7 days). The nature, severity, and duration of these eye injuries suggest the potential to cause human injury. The purpose of ocular toxicity labeling is to communicate potential hazards of chemicals and products to workers and consumers so that appropriate measures can be taken to avoid accidental or inadvertent contact with the eye. In addition, ocular safety labels provide the necessary first aid measures that should be taken in the event of accidental exposures.

Among the fundamental principles agreed upon by participants establishing the GHS was the assurance that "the level of protection offered to workers, consumers, the general public and the environment should not be reduced as a result of harmonizing the classification and labeling systems" (UN 2007). ICCVAM has conducted technical analyses to support the development of appropriate recommendations for GHS options that would continue to provide at least equivalent protection as current U.S. ocular hazard classification and labeling requirements. ICCVAM recommends that U.S. agencies consider the GHS ocular hazard classification criteria and categories and the level of protection they provide compared to current U.S. hazard classification systems.

Federal law requires agencies to determine that new test methods recommended by ICCVAM generate data that are at least equivalent to that generated by test methods they currently require or recommend for hazard identification purposes. Given that the BCOP test method does not identify eye irritation hazards when using the EU or GHS hazard classification systems that are currently identified using U.S. hazard identification and classification requirements (EPA and FHSA), ICCVAM cannot recommend using the BCOP test method as a screening test to identify substances not labeled as irritants for the GHS classification system. ICCVAM will revisit recommendations for the BCOP test method based on any updates to the GHS eye hazard classification criteria that may occur as a result of the NICEATM analyses.

Identification of Reversible Eye Irritation Hazard Categories

Based on an evaluation of available data and corresponding performance (accuracy and reliability), ICCVAM concludes that the BCOP test method is **not** recommended to identify moderate and mild ocular irritants as defined by the EPA, EU, and GHS classification systems (EPA 2003; EU 2001; UN 2007).[4]

[4] The FHSA ocular hazard category that is assigned based on results from the Draize rabbit eye test does not distinguish between mild and moderate ocular irritants.

Table 2-1 *In Vivo* Ocular Lesions from False Negative Substances in the BCOP Test Method Using the EPA Classification System

Compound	N	*In Vivo* Scores[1]			
		Corneal Opacity: Score (day cleared)	Iris: Score (day cleared)	Conjunctival Redness: Score (day cleared)	Chemosis: Score (day cleared)
Dimethylbiquanide	3	N = 1 1(2) N = 1 1(3)	N = 1 1(2)	N = 2 2(3)	N = 2 2(1)
EDTA	3	N = 1 1(3)	N = 2 1(1)	N = 3 2(2)	N = 1 2(1) N = 1 2(2) N = 1 3(2)
Magnesium carbonate	3	N = 1 1(2) N = 1 1(3)	None	None	None
Polyalkenylsuccinate ester/amine salt	6	N = 2 1(2)	None	N = 1 2(6) N = 3 2(2) N = 1 3(2) N = 1 3(6)	N = 1 2(1) N = 1 2(2)
Compound I	6	N = 1 1(2)	None	None	None
Iminodibenzyl	3	N = 3 1(2)	None	None	None
Methylcyclopentane	6	None	None	N = 1 2(3)	None
Tween 20	4	None	None	N = 2 2(2)	None

Abbreviations: N = number of animals.

[1] The following scores are considered positive: CO or IR ≥1 or CC or CR ≥2. Therefore, CO or IR scores of 0 and CC or CR scores of ≤1 are considered cleared.

Evaluation as a Screening Test to Identify Ocular Corrosives and Severe Irritants

In the original ICCVAM evaluation of the BCOP test method as a screening test to identify substances as ocular corrosives and severe irritants, overall accuracy was 79% (113/143) to 81% (119/147), false positive rates were 19% (20/103) to 21% (22/103), and false negative rates were 16% (7/43) to 25% (10/40) depending on the hazard classification system (i.e., EPA Category I, EU R41, GHS Category 1).

Based on the current updated BCOP validation database, which has increased from 145 to 211 substances, overall accuracy of the BCOP test method as a screening test to identify substances as ocular corrosives and severe irritants is 77% (91/118) to 79% (148/187) depending on the hazard classification system (i.e., EPA Category I, EU R41, GHS Category 1).[5] The false positive rate is 23% (29/124) to 24% (29/122), and false negative rates are 15% (10/65) to 21% (7/33) depending on the hazard classification system used. Based on the similar performance statistics of the current and the original databases, the ICCVAM recommendation for the use of the BCOP test method to identify substances as ocular corrosives and severe irritants remains unchanged:

> There are sufficient data to support the use of the BCOP test method, in appropriate circumstances and with certain limitations, as a screening test to identify substances as ocular corrosives and severe irritants (i.e., EPA Category I, GHS Category 1, EU R41) in a tiered-

[5] The FHSA ocular hazard category that is assigned based on results from the Draize rabbit eye test does not distinguish between ocular corrosives/severe irritants and less severe irritants. For this reason, an evaluation of the BCOP test method as a screening test to identify ocular corrosives/severe irritants using the FHSA classification system is not possible.

testing strategy, as part of a weight-of-evidence approach. In a tiered-testing strategy, when a positive result is obtained in an appropriately validated *in vitro* test, a test substance may be classified as an ocular hazard without testing in rabbits. A substance that tests negative in the *in vitro* ocular toxicity test would need to be tested in the *in vivo* ocular test to identify possible *in vitro* false negatives and to identify moderate and mild ocular irritants (ICCVAM 2006e).

Independent Peer Review Panel Conclusions and Recommendations
The Panel concluded that the available data and test method performance support the ICCVAM draft recommendations that the BCOP test method is not recommended to distinguish substances from all hazard categories as defined by the EPA, EU, and GHS classification systems. The Panel agreed with ICCVAM that the BCOP test method continue to be recommended as a screening test for severe irritants. The Panel also concluded that the BCOP test method can be used as a screening test to distinguish substances not labeled as irritants from all other hazard categories when results are to be used for EU or GHS hazard classifications. *It should be noted that this recommendation preceded the NICEATM evaluation of the GHS classification system and, therefore, this information was not taken into consideration.* However, like ICCVAM, the Panel concluded that, because of the significant lesions associated with 50% (4/8) of the EPA Category III substances that tested as false negative, the BCOP test method cannot be recommended as a screening test to identify EPA Category IV substances.

2.1.2 ICCVAM Recommendations: BCOP Test Method Protocol

For use of the BCOP test method as a screening test to identify substances as ocular corrosives and severe irritants (i.e., EPA Category I, EU R41, GHS Category 1), ICCVAM recommends using the updated ICCVAM BCOP test method protocol that is included as an appendix to this report (**Appendix B**). In addition, all future studies intended to further characterize the usefulness and limitations of the BCOP test method should be conducted using this protocol.

Independent Peer Review Panel Conclusions and Recommendations
While the BCOP test method protocol was previously reviewed for use in identifying ocular corrosives and severe irritants, the Panel emphasized the importance of protocol elements. They emphasized that use of this protocol to identify mild/moderate ocular irritants in future studies should include (1) methods for harvest and storage of eyes, (2) timeframe from harvest to use of eyes, (3) consistent animal age, (4) screening for existing corneal lesions prior to use, (5) concurrent positive and negative controls, (6) inclusion of an untreated negative control, and (7) refinement of histopathological methodology.

2.1.3 ICCVAM Recommendations: Future Studies for the BCOP Test Method

To further the use of this test method and to evaluate the use of the BCOP test method as a potential replacement for the Draize rabbit eye test or for the identification of mild and moderate ocular irritants (i.e., EPA Category II, III; EU R36; GHS Category 2A, 2B) and substances not labeled as irritants (i.e., EPA Category IV; EU Not Labeled; FHSA Not Labeled; GHS Not Classified), ICCVAM recommends additional studies be considered and undertaken.

- Additional optimization studies/evaluations should be conducted in an attempt to improve the correct classification of mild and moderate ocular irritants and substances not labeled as irritants. After optimization, additional studies to further assess the reliability and accuracy of the test method are recommended.
- ICCVAM recommends that a histopathological evaluation of the corneal tissue, using standardized procedures, be included when the BCOP test method is conducted. Such data will allow for development of decision criteria and future assessments on the usefulness of

this endpoint for classifying and labeling substances, especially those that may otherwise produce borderline or false negative results.

- ICCVAM encourages users to provide all data that are generated from future studies. They could be used to further characterize the usefulness and limitations of the BCOP test method for the identification of all ocular hazard categories.

Independent Peer Review Panel Conclusions and Recommendations

The Panel concluded that the available data support the ICCVAM draft recommendations. The Panel encouraged continued test method development and refinement of the protocol to achieve more accurate classification of mild and moderate irritants. The Panel also recommended that problematic chemical classes within these hazard categories be identified in order to determine if improved performance could be achieved by restricting the applicability domain.

2.1.4 ICCVAM Recommendations: Performance Standards for the BCOP Test Method

Based on the available data and associated performance described in the final ICCVAM BRD (**Appendix C**), ICCVAM recommends that the development of performance standards for the BCOP test method is not warranted at this time.

2.2 Validation Status of the BCOP Test Method

The following is a synopsis of the information in the final ICCVAM BRD (**Appendix C**), which reviews the available data and information for the BCOP test method. The ICCVAM BRD describes the current validation status of the BCOP test method, including what is known about its reliability and accuracy, the scope of substances tested, and standardized protocols for the validation study.

2.2.1 Test Method Description

The BCOP test method is an *in vitro* eye irritation test method using isolated bovine corneas that are byproducts from processing plants. In the BCOP test method, opacity is determined by the amount of light transmitted through the cornea, and permeability is determined by the amount of sodium fluorescein dye that passes through all corneal cell layers. Both measurements are used to calculate an IVIS, which is used to assign an *in vitro* irritancy classification to predict the *in vivo* ocular irritation potential of a test substance.

2.2.2 Validation Database

An online literature search conducted in support of the evaluation of the validation status of the BCOP test method identified four publications containing BCOP test method results. However, none of these publications included raw data or *in vivo* reference data, or they included data cited from earlier studies that were already included in the validation database. Accordingly, these were not added to the database. The results from the BCOP test method for 66 antimicrobial cleaning products (AMCPs) were obtained from a submission to ICCVAM that describes a non-animal approach for evaluating eye irritation potential and labeling requirements for AMCPs. Therefore, the previous validation database for the BCOP test method (ICCVAM 2006a) was updated to include BCOP data for the 66 AMCPs. The updated BCOP validation database contains 211 substances, representing a wide variety of chemical and product classes, and including 135 commercial products or formulations.

Detailed *in vivo* data were necessary to calculate the appropriate EPA, EU, FHSA, and GHS ocular hazard classifications (EPA 2003; EU 2001; FHSA 2005; UN 2007) (**Appendix C**). These data include cornea, iris, and conjunctiva scores for each animal at 24, 48, and 72 hours and/or assessment of the presence or absence of lesions at 7, 14, and 21 days. Thus, some of the test substances for which there was only limited *in vivo* data could not be used for evaluating test method accuracy and reliability. Additionally, because the FHSA classification system is based on a sequential testing strategy that uses up to 18 animals, only a small percentage of the substances in the test method

databases would be classifiable if the FHSA criteria were strictly applied. Therefore, to maximize the number of substances included in these analyses, two separate "proportionality" criteria were applied for the purpose of assigning an FHSA classification. Based on the minimum number of positive animals needed to identify a substance as an irritant using a single 6-animal test in the FHSA sequential testing strategy, a 67% threshold for positive responding animals was used (i.e., at least 2/3 or 4/6 positive animals) to assign an irritant classification. Alternatively, based on the minimum number of positive animals needed (4/18 [22%]) to identify a substance as an irritant when three 6-animal tests are required in the sequential testing strategy, a 20% threshold for positive responding animals was used to assign an irritant classification.

2.2.3 Test Method Accuracy

Identification of All Ocular Hazard Categories

The ability of the BCOP test method to identify all categories of ocular irritation potential was evaluated for the EPA, EU, and GHS classification systems (EPA 2003; EU 2001; UN 2007).[6] As indicated in **Table 2-2**, overall correct classifications ranged from 49% (91/187) to 55% (102/187), depending on the hazard classification system used when evaluating the entire database. Using alternative decision criteria to identify ocular corrosives and severe irritants (i.e., IVIS ≥ 75 [used in the AMCP submission protocol] instead of IVIS ≥ 55.1 [as per the ICCVAM-recommended BCOP protocol]) did not improve test method performance.

Distinguishing Substances Not Labeled as Irritants from All Other Hazard Categories

The ability of the BCOP test method to distinguish substances not labeled as irritants (i.e., EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified) from all other ocular hazard categories (i.e., EPA Category I, II, III; EU R41, R36; FHSA Irritant; GHS Category 1, 2A, 2B), as defined by the EPA, EU, FHSA, and GHS classification systems (EPA 2003; EU 2001; FHSA 2005; UN 2007) was also evaluated.

As indicated in **Table 2-3**, overall accuracy for the identification of substances not labeled as irritants (i.e., EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified) from all other categories ranged from 64% (76/118) to 83% (148/179, 155/107, or 161/194) depending on the hazard classification system used. While false positive rates were high (53% [24/45 or 25/47] to 70% [63/90]) depending on the hazard classification system used), the false negative rates were low (0% [0/54 or 0/97] to 6% [8/142]) depending on the hazard classification system used. All eight of the false negative substances for the EPA classification system were EPA Category III (i.e., ocular injuries to the cornea and/or iris [inside the eye] and/or conjunctival injuries that persisted more than 24 hours but less than 7 days) based on Draize rabbit eye test data (**Table 2-1**). This included three substances (38% [3/8]) that were classified as EPA eye irritants based on at least one rabbit with corneal lesions and opacity that did not resolve until day 3 of the study. A fourth substance was classified as an EPA eye irritant based on all six rabbits with conjunctival redness scores of 3 (*producing diffuse, crimson color of the conjunctiva, individual blood vessels not easily discernable*). The conjunctival redness scores for two of these animals did not recover to a score of 1 (s*ome blood vessels definitely hyperemic*) until day 6 of the study. The conjunctival redness scores for the remaining four rabbits recovered to a score of 1 on day 2 of the study.

[6] The FHSA ocular hazard category that is assigned based on results from the Draize rabbit eye test does not distinguish between ocular corrosives/severe irritants and less severe irritants. For this reason, an evaluation of the BCOP test method to identify all ocular hazard categories using the FHSA classification system is not possible.

Table 2-2 Evaluation of the Performance of the BCOP Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by EPA, EU, and GHS Classification Systems[1]

Severe using ≥55.1											
	Overall Correct Classification	Severe[2]		Moderate[3]			Mild[4]			Not Labeled[5]	
		Actual	Under	Over	Actual	Under	Over	Actual	Under	Over	Actual
GHS	49% (91/187)	85% (55/65)	15% (10/65)	62% (16/26)	27% (7/26)	11% (3/26)	67% (4/6)	33% (2/6)	0% (0/6)	70% (63/90)	30% (27/90)
EPA	55% (102/187)	84% (53/63)	16% (10/63)	50% (11/22)	32% (7/22)	18% (4/22)	50% (28/57)	36% (21/57)	14% (8/57)	53% (24/45)	47% (21/45)
EU	50% (59/118)	79% (26/33)	21% (7/33)	48% (10/21)	52% (11/21)	0% (0/21)	NA	NA	NA	66% (42/64)	34% (22/64)
Severe using ≥75											
		Severe		Moderate			Mild			Not Labeled	
		Actual	Under	Over	Actual	Under	Over	Actual	Under	Over	Actual
GHS	50% (94/187)	78% (51/65)	22% (14/65)	31% (8/26)	54% (14/26)	15% (4/26)	67% (4/6)	33% (2/6)	0% (0/6)	70% (63/90)	30% (27/90)
EPA	49% (92/187)	78% (49/63)	22% (14/63)	36% (8/22)	45% (10/22)	19% (4/22)	47% (27/57)	39% (22/57)	14% (8/57)	53% (24/45)	47% (21/45)
EU	51% (60/118)	73% (24/33)	27% (9/33)	29% (6/21)	67% (14/21)	4% (1/21)	NA	NA	NA	66% (42/64)	34% (22/64)

Abbreviations: BCOP = bovine corneal opacity and permeability; EPA = U.S. Environmental Protection Agency; EU = European Union; GHS = Globally Harmonized System; NA = not applicable.

[1] EPA classification system (EPA 2003); EU classification system (EU 2001); GHS classification system (UN 2007).

[2] Severe = GHS Category 1; EPA Category I; EU R41.

[3] Moderate = GHS Category 2A; EPA Category II; EU R36.

[4] Mild = GHS Category 2B; EPA Category III.

[5] Not Labeled = GHS Not Classified; EPA Category IV; EU Not Labeled.

Table 2-3 Accuracy of the BCOP Test Method for Distinguishing Substances Not Labeled as Irritants[1] from All Other Irritant Classes

	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
GHS	187	66	124/187	100	97/97	30	27/90	70	63/90	0	0/97
EPA	187	83	155/187	94	134/142	47	21/45	53	24/45	6	8/142
EU	118	64	76/118	100	54/54	34	22/64	66	42/64	0	0/54
FHSA-20%	194	83	161/194	95	139/147	47	22/47	53	25/47	5	8/147
FHSA-67%	179	83	148/179	95	126/132	47	22/47	53	25/47	5	6/132

Abbreviations: BCOP = bovine corneal opacity and permeability; EPA = U.S. Environmental Protection Agency; EU = European Union; FHSA = U.S. Federal Hazardous Substances Act; GHS = Globally Harmonized System; N = number of substances included in this analysis; No. = data used to calculate the percentage.

[1] GHS classification system (UN 2007): Not Classified vs. Category 1/2A/2B.
EPA classification system (EPA 2003): Category IV vs. Category I/II/III.
EU classification system (EU 2001): Not Labeled vs. R41/R36.
FHSA classification system (FHSA 2005): Not Labeled vs. Irritant.

2.2.4 Test Method Reliability

Interlaboratory Reproducibility

Quantitative and qualitative evaluations of the BCOP test method reliability have been conducted previously (ICCVAM 2006a). However, additional qualitative analyses of interlaboratory reproducibility were conducted to evaluate the extent of agreement of BCOP hazard classifications among the laboratories participating in the three interlaboratory validation studies (Balls et al. 1995; Gautheron et al. 1994; Southee 1998). As was done for the accuracy evaluation, these qualitative evaluations of reproducibility were based on (1) the use of the BCOP test method for identifying all ocular hazard categories according to the EPA, EU, or GHS systems and (2) the use of the BCOP test method to distinguish substances not labeled as irritants (i.e., EPA Category IV, EU Not Labeled, GHS Not Classified) from all other ocular hazard categories (i.e., EPA Category I, II, III; EU R41, R36; GHS Category 1, 2A, 2B). Given that the performance of the BCOP test method was similar for the EPA and FHSA classification systems, additional reliability analyses were not conducted for the FHSA classification system.

Using the first approach (i.e., identifying all ocular hazard categories) among the three interlaboratory studies for the Balls et al. (1995) study, there was 100% agreement among the five laboratories for a majority of the Draize ocular corrosives and severe irritants based on all three classification systems, whether they were correctly identified or underclassified by the BCOP test method (e.g., for the GHS system, there was 100% agreement for 88% [15/17] of the correctly identified Category 1 substances). There was also 100% agreement among the five laboratories for 100% (10/10) of the overpredicted Not Labeled substances and for at least 50% (2/4) of the correctly identified Not Labeled substances.

For the Gautheron et al. (1994) study, there was 100% agreement among the 11 laboratories for a majority of the Draize ocular corrosives and severe irritants based on all three classification systems, whether they were correctly identified or underclassified by the BCOP test method (e.g., for the GHS system, there was 100% agreement for 67% [4/6] of the correctly identified Category 1 substances). There was also 100% agreement among the 11 laboratories for a majority of the overpredicted Not Labeled substances (e.g., for the EU system, there was 100% agreement for 54% [7/13] of the

correctly identified Not Labeled substances) and for a majority of the incorrectly identified Not Labeled substances (e.g., for the EU system, there was 100% agreement for 91% [21/23] of the correctly identified substances).

For the Southee (1998) study, there was 100% agreement among the three laboratories for all of the ocular corrosives and severe irritants based on all three classification systems, whether they were correctly identified or underclassified by the BCOP test method (e.g., for the GHS system, there was 100% agreement for 100% [4/4] of the Draize ocular corrosives and severe irritants). There was also 100% agreement among the two correctly identified Not Labeled substances.

Using the second approach (i.e., distinguishing substances not labeled as irritants from all other ocular hazard categories) for the Balls et al. (1995) study, there was 100% agreement for 92% (55/60) to 93% (56/60) of the substances tested *in vitro*, depending on the classification system used. All five laboratories were in 100% agreement on the classification of 50% (2/4) of Not Labeled substances and 94% (32/34) to 96% (48/50) of all other irritant class substances, depending on the classification system used.

For the Gautheron et al. (1994) study, there was 100% agreement among the eleven laboratories for 65% (34/52) of the substances tested *in vitro*, for all classification systems. There was 100% agreement among the laboratories on the classification of 83% (10/12) to 87% (27/31) of all other irritant class substances, depending on the classification system used.

There was 100% agreement among the three laboratories in the Southee (1998) study for 88% (14/16) of the substances tested *in vitro*, for all classification systems. All three laboratories were in 100% agreement on the classification of 100% (2/2) Not Labeled substances and 90% (9/10) to 92% (11/12) of all other irritant class substances, depending on the classification system used.

As stated above, the final ICCVAM BRD (**Appendix C**) provides a comprehensive summary of the current validation status of the BCOP test method, including what is known about its reliability and accuracy, and the scope of substances tested. Raw data for the BCOP test method will be maintained for future use, so that these performance statistics may be updated as additional information becomes available.

2.2.5 Animal Welfare Considerations

The BCOP test method refines animal use. Because these animals are being humanely processed for nonlaboratory purposes, the testing procedure inflicts no additional pain or distress. Substances that are identified as corrosive or severe irritants *in vitro* are excluded from *in vivo* testing.

The BCOP test method can also reduce animal use because the test method utilizes animal species routinely raised as a food source in large numbers and thus replaces the need for laboratory animals.

3.0 The Cytosensor Microphysiometer Test Method

A number of *in vitro* test systems that have been proposed as alternatives to the Draize rabbit eye test rely on cell death as an endpoint. However, reversible cell changes may provide more appropriate endpoints for the assessment of ocular irritation potential. Good correlations have been reported between results obtained from the CM test method and *in vivo* eye irritancy data. The method is noninvasive, and thus allows the determination of recovery of the cells from the toxic insult. The CM test method measures the rate of extracellular acidification of populations of living cells maintained in flow chambers. After establishing a baseline acidification rate for each set of cells and measuring the new rates subsequent to each sample addition, the concentration of test material (w/v%) required to reduce the acidification rate to 50% is computed by interpolation between the rate data points spanning the 50% response level. This value is termed the MRD_{50} and is the endpoint for the test.

ICCVAM evaluated the validation status of the CM test method, which was not part of the ICCVAM 2006 evaluation, as an *in vitro* alternative to the Draize rabbit eye test for identifying ocular corrosives and severe irritants (i.e., those that induce irreversible ocular damage; EPA Category I, EU R41, GHS Category 1) and substances not labeled as irritants (i.e., EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified) according to the EPA, EU, FHSA, and GHS classification systems (EPA 2003; EU 2001; FHSA 2005; UN 2007).

3.1 ICCVAM Recommendations

3.1.1 ICCVAM Recommendations: Test Method Usefulness and Limitations

Evaluation as a Screening Test to Identify Substances Not Labeled as Irritants
ICCVAM concludes that the accuracy and reliability of the CM test method are sufficient to support its use as a screening test to distinguish water-soluble surfactant chemicals and certain types of surfactant-containing formulations (e.g., cosmetics and personal care product formulations, but not pesticide formulations) that are not labeled as irritants (i.e., EPA Category IV, EU Not Labeled, FHSA Not Labeled) from all other hazard categories (i.e., EPA Category I, II, III; EU R41, R36; FHSA Irritant) when results are to be used specifically for hazard classification and labeling purposes under the EPA, EU and FHSA classification systems (EPA 2003; EU 2001; FHSA 2005). Until the issues associated with the GHS classification system are further discussed (see "BCOP Test Method Usefulness and Limitations"), ICCVAM is deferring final recommendations on the usefulness and limitations of using the CM test method as a screening test to identify substances not labeled as irritants according to the GHS classification system.

When the CM test method was used to distinguish substances not labeled as irritants among the database of 53 surfactant-containing substances, the false negative rate ranged from 0% (0/27) to 2% (1/47) depending on the hazard classification system used. The one false negative substance based on *in vivo* data was EPA Category III or FHSA Irritant. For this substance, six test animals were included. One test animal had no observable effects, three test animals had conjunctival redness (score = 1), and two test animals had corneal opacity (score = 1) that cleared after one day.

When the CM test method was used to distinguish substances not labeled as irritants among the database of 29 water-soluble nonsurfactant substances and formulations, the false negative rate ranged from 24% (5/21) to 40% (8/20) depending on the hazard classification system used. Because of these high false negative rates, the CM test method is **not** recommended as a screening test to distinguish substances not labeled as irritants among these types of substances.

Evaluation as a Screening Test to Identify Ocular Corrosives and Severe Irritants
ICCVAM recommends that the CM test method can be used as a screening test to identify water-soluble substances (i.e., water-soluble surfactants, surfactant-containing formulations, and nonsurfactants) as ocular corrosives and severe irritants (i.e., EPA Category I, EU R41, GHS

Category 1[7]) in a tiered-testing strategy, as part of a weight-of-evidence approach.[8] A substance that tests negative with the CM test method would need to be tested in another test method that is capable of identifying possible *in vitro* false negative ocular corrosives and severe irritants and to distinguish between moderate and mild ocular irritants. Currently, the Draize rabbit eye test is the only test method capable of making such a distinction.

Given that the CM test method (INVITTOX Protocol Number 102) is proposed for use as a screening test to identify both ocular corrosives and severe irritants and substances not labeled as irritants, users may want to consider using the CM test method prior to another *in vitro* ocular test method for testing these types of substances. However, testing in another test method would be necessary for certain substances not identified with the CM test method: (1) water-soluble substances that are not identified as ocular corrosives and severe irritants or (2) water-soluble surfactant chemicals and specific types of surfactant-containing formulations that are not identified as substances not labeled as irritants. The other test method must be capable of correctly classifying substances into each of the four hazard classification categories for the EPA or GHS classification systems. Currently, the only test method accepted for these purposes is the Draize rabbit eye test. Because of the high false positive rate (50% [3/6] to 69% [18/26] depending on the hazard classification system used) for substances not labeled as irritants, users may not want to use the CM test method if the intention is to identify substances not labeled as irritants first.

Independent Peer Review Panel Conclusions and Recommendations

The Panel concluded that the CM test method is recommended as a screening test to identify water-soluble surfactant substances as ocular corrosives and severe irritants and substances not labeled as irritants in a tiered-testing strategy, as part of a weight-of-evidence approach. However, major concerns are the continued availability of the instrument used to conduct the CM test method, and what new manufacturing processes, including the subsequent required revalidation, might mean to already existing CM test method data.

When using the CM test method to identify water-soluble surfactant substances as substances not labeled as irritants, the false negative rate for the EU, FHSA-67%, and GHS systems was 0% (0/27 or 0/28). For the EPA system it was 2% (1/46). Therefore, the CM test method was recommended for such testing purposes. The Panel recommended that further studies using the CM test method are needed, in particular for EPA Categories III and IV, and that the available data restrict the applicability domain of the CM test method to water-soluble surfactants and surfactant-based formulations (e.g., cosmetics and personal care products).

3.1.2 ICCVAM Recommendations: CM Test Method Protocol

For use of the CM test method as a screening test to identify water-soluble substances as ocular corrosives and severe irritants (i.e., EPA Category I, EU R41, GHS Category 1), or to identify substances not labeled as irritants (i.e., EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified), ICCVAM recommends using the ICCVAM CM test method protocol that is included as an appendix to this report (**Appendix B**). In addition, all future studies intended to further characterize the usefulness and limitations of the CM test method should be conducted using this protocol.

---

[7] The FHSA ocular hazard category that is assigned based on results from the Draize rabbit eye test does not distinguish between ocular corrosives/severe irritants and less severe irritants. For this reason, an evaluation of the CM test method as a screening test to identify ocular corrosives/severe irritants using the FHSA classification system is not possible.

[8] The ECVAM Scientific Advisory Committee (ESAC) has also recommended the CM test method for this purpose and for this limited applicability domain (ESAC 2009; **Appendix K**).

Independent Peer Review Panel Conclusions and Recommendations
The Panel concluded that critical aspects of the CM test method had been adequately justified and described and that the protocol was sufficiently detailed. The Panel supported the use of the recommended protocol for future studies to further characterize the usefulness and limitations of the CM test method. However, they expressed concern that the CM test method is unlikely to be widely used because manufacture of the instrument required to conduct the test method has been discontinued.

3.1.3 ICCVAM Recommendations: Future Studies for the CM Test Method

To expand the applicability domain of the CM test method for the identification of ocular corrosives and severe irritants (i.e., EPA Category I, GHS Category 1, EU R41) and substances not labeled as irritants (i.e., EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified), ICCVAM recommends additional studies be considered and undertaken.

- ICCVAM recommends that these substances be selected from the ICCVAM-recommended reference substances for validation of *in vitro* ocular safety test methods for the evaluation of ocular corrosives and severe irritants[9] in order to provide for a more direct assessment of the CM test method's utility as a screening test for identifying ocular corrosives and severe irritants. Similarly, a reference set could also be selected from this list for the purposes of assessing the utility of the CM test method as a screening test for identifying substances not labeled as irritants.
- ICCVAM recommends that future optimization studies be directed towards increasing the performance of the CM test method for identifying all categories of ocular irritancy hazard classification according to the EPA, EU, or GHS hazard classification systems. This will require that an increased number of substances in the moderate and mild ocular irritant categories (i.e., EPA Category II, III; EU R36; GHS Category 2A, 2B) be identified and tested.
- ICCVAM encourages users to provide all data that are generated from future studies, as they could be used to further characterize the usefulness and limitations of the CM test method for the identification of all ocular hazard categories.

Independent Peer Review Panel Conclusions and Recommendations
The Panel concluded that additional studies should be conducted to further characterize the usefulness and limitations of the CM test method for use as a screening test for identifying ocular corrosives and severe irritants or substances not labeled as irritants. Because the CM test method is limited to testing water-soluble surfactants and certain types of surfactant formulations, the Panel recommended that a range of surfactant concentrations should be tested because surfactants form micelles at higher concentrations, which reduce the number of surfactant molecules available to react with the target tissue.

3.1.4 ICCVAM Recommendations: Performance Standards for the CM Test Method

Based on the available data and associated performance described in the redacted ECVAM CM BRD (**Appendix D**), ICCVAM recommends that the development of performance standards for the CM test method is not warranted at this time.

3.2 Validation Status of the Cytosensor Microphysiometer Test Method

The following is a synopsis of the information for three of the peer-reviewed publications (Balls et al. 1995, Gettings et al. 1996, Brantom et al. 1997) referenced in the redacted ECVAM CM BRD (**Appendix D**) and utilized by ICCVAM in its review. The redacted ECVAM CM BRD describes the

[9] http://iccvam.niehs.nih.gov/methods/ocutox/ivocutox/ocu_tmer.htm

current validation status of the CM test method, including what is known about its reliability and accuracy, the scope of substances tested, and standardized protocols for the validation study.

3.2.1 Test Method Description

The CM test method estimates the metabolic rate (i.e., glucose utilization rate) of cells by measuring the rate of excretion of acid byproducts and resulting decrease in pH of the surrounding medium in an enclosed chamber. The rate of change in pH per unit time becomes the metabolic rate of the population. If a test material causes cytotoxicity to this population of cells it is assumed that the metabolic rate will fall. Although the metabolic rate is the physical parameter that is measured with the CM test method, the magnitude of the metabolic rate itself is not directly related to eye irritation potential. Rather, the reduction of the metabolic rate to 50% of its basal rate is the parameter used to measure the impact of the test article on the test system (L929 cells in almost all cases). The CM test method exposes a population of cells to increasing concentrations of the test article (diluted in medium). The exposure follows a 3-step process. The first step is the exposure to the diluted test article, the second is the test article rinse-out, and the third is the measurement of the metabolic activity. This means that the impact of the exposure is measured immediately, and then a subsequent exposure is performed until the highest testable concentration has been used or the population of cells is severely damaged and the metabolic rate has declined to effectively zero. From the concentration response curve, the concentration that leads to a 50% decline in the metabolic rate of the population (the MRD_{50}) is calculated. The MRD_{50} values are used to compare test materials and provide a measure of ocular irritancy potential.

3.2.2 Validation Database

Data on 53 water-soluble surfactant and surfactant-containing formulations were provided in the redacted ECVAM CM BRD (**Appendix D**), where data from the Draize rabbit eye test were also available to assess the accuracy of the CM test method. The database of 53 water-soluble surfactants tested in the CM test method included 21 surfactant chemicals and 32 surfactant-containing formulations tested across seven different laboratories.

The nonsurfactant substances database (n = 29) consisted of 27 water-soluble nonsurfactant chemicals, which included a range of chemical classes (e.g., acids, alcohols, alkalis, and ketones), and two nonsurfactant formulations (n = 2) tested in seven laboratories.

3.2.3 Test Method Accuracy

Distinguishing Substances Not Labeled as Irritants from All Other Hazard Categories
NICEATM evaluated the CM test method's ability to distinguish substances not labeled as irritants (EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified) from all other hazard categories (EPA Category I, II, III; EU R41, R36; FHSA Irritant; GHS Category 1, 2A, 2B), as defined by the EPA, EU, FHSA, and GHS classification systems (EPA 2003; EU 2001; FHSA 2005; UN 2007) among the water-soluble surfactants and surfactant-containing formulations.

As indicated in **Table 3-1**, overall accuracy for the identification of substances not labeled as irritants (i.e., EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified) from all other categories for the water-soluble surfactants and surfactant-containing formulations ranged from 66% (35/53) to 93% (43/46) depending on the hazard classification system used. The false negative rate ranged from 0% (0/27, 0/28, or 0/40) to 2% (1/46 or 1/47) depending on the hazard classification system used. The one false negative in both the EPA and the FHSA-20% classification systems was classified as Category III and Irritant, respectively, based on Draize rabbit eye test data. For this substance, six rabbits were included in the *in vivo* test. One rabbit had no observable effects, three rabbits had conjunctival redness (score = 1), and two rabbits had corneal opacity (score = 1) that cleared after one day.

The ability of the CM test method to distinguish substances not labeled as irritants (i.e., EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified) from all other hazard categories (i.e., EPA Category I, II, III; EU R41, R36; FHSA Irritant; GHS Category 1, 2A, 2B), as defined by the EPA, EU, FHSA, and GHS classification systems (EPA 2003; EU 2001; FHSA 2005; UN 2007) among the water-soluble nonsurfactant substances was also evaluated.

As indicated in **Table 3-2**, overall accuracy for the identification of substances not labeled as irritants (i.e., EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified) from all other categories for the water-soluble nonsurfactant substances ranged from 63% (15/24) to 76% (22/29) depending on the hazard classification system used. The false negative rate ranged from 24% (5/21) to 40% (8/20) depending on the hazard classification system used. Eight substances were false negative when using the EPA, GHS, and FHSA classification systems. In the EPA system, they were classified *in vivo* as Category 1 (n = 1), Category II (n = 3) and Category III (n = 4). In the GHS system, they were classified *in vivo* as Category 1 (n = 1) and Category 2A (n = 7). For the FHSA system, they were classified *in vivo* as Irritant.

Table 3-1 Accuracy of the CM Test Method for Distinguishing Substances Not Labeled as Irritants[1] from All Other Irritant Classes for Surfactant-Containing Substances

	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
GHS	53	68	36/53	100	28/28	32	8/25	68	17/25	0	0/28
EPA	52	92	48/52	98	45/46	50	3/6	50	3/6	2	1/46
EU	53	66	35/53	100	27/27	31	8/26	69	18/26	0	0/27
FHSA-20%	53	92	49/53	98	46/47	50	3/6	50	3/6	2	1/47
FHSA-67%	46	93	43/46	100	40/40	50	3/6	50	3/6	0	0/40

Abbreviations: CM = Cytosensor Microphysiometer; EPA = U.S. Environmental Protection Agency; EU = European Union; FHSA = U.S. Federal Hazardous Substances Act; GHS = Globally Harmonized System; N = number of substances included in this analysis; No. = data used to calculate the percentage.

[1] GHS classification system (UN 2007): Not Classified vs. Category 1/2A/2B.
EPA classification system (EPA 2003): Category IV vs. Category I/II/III.
EU classification system (EU 2001): Not Labeled vs. R41/R36.
FHSA classification system (FHSA 2005): Not Labeled vs. Irritant.

Table 3-2 Accuracy of the CM Test Method for Distinguishing Substances Not Labeled as Irritants[1] from All Other Irritant Classes for Nonsurfactant Substances

	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
GHS	25	64	16/25	62	13/21	75	3/4	25	1/4	38	8/21
EPA	29	66	19/29	67	16/24	60	3/5	40	2/5	33	8/24
EU	29	76	22/29	76	16/21	75	6/8	25	2/8	24	5/21
FHSA-20%	25	64	16/25	62	13/21	75	3/4	25	1/4	38	8/21
FHSA-67%	24	63	15/24	60	12/20	75	3/4	25	1/4	40	8/20

Abbreviations: CM = Cytosensor Microphysiometer; EPA = U.S. Environmental Protection Agency; EU = European Union; FHSA = U.S. Federal Hazardous Substances Act; GHS = Globally Harmonized System; N = number of substances included in this analysis; No. = data used to calculate the percentage.

[1] GHS classification system (UN 2007): Not Classified vs. Category 1/2A/2B.
EPA classification system (EPA 2003): Category IV vs. Category I/II/III.
EU classification system (EU 2001): Not Labeled vs. R41/R36.
FHSA classification system (FHSA 2005): Not Labeled vs. Irritant.

Distinguishing Ocular Corrosives and Severe Irritants from All Other Hazard Categories
The ability of the CM test method to distinguish ocular corrosives and severe irritants (i.e., EPA Category I, EU R41, GHS Category 1) from all other ocular hazard categories (i.e., EPA Category II, III, IV; EU R36, Not Labeled; GHS Category 2A, 2B, Not Classified) as defined by the EPA, EU, and GHS classification systems (EPA 2003; EU 2001; UN 2007)[10] was evaluated among the water-soluble surfactants and surfactant-containing formulations.

As indicated in **Table 3-3**, overall accuracy for the identification of ocular corrosives and severe irritants (i.e., EPA Category I, EU R41, GHS Category 1) from all other categories for surfactant-containing substances ranged from 85% (44/52) to 94% (50/53) depending on the hazard classification system used. The false positive rates ranged from 3% (1/30) to 10% (3/29) depending on the hazard classification system used. The three false positives when using the EPA classification system are classified as Category II (n = 2) or III (n = 1) based on Draize rabbit eye test data. The one false positive when using the GHS and EU classification systems is Not Classified and Not Labeled, respectively, based on Draize rabbit eye test data.

Table 3-3 Accuracy of the CM Test Method for Distinguishing Corrosives/Severe Irritants[1] from All Other Irritant Classes for Surfactant-Containing Substances

	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
GHS	53	94	50/53	91	21/23	97	29/30	3	1/30	9	2/23
EPA	52	85	44/52	78	18/23	90	26/29	10	3/29	22	5/23
EU	53	89	47/53	81	21/26	96	26/27	4	1/27	19	5/26

Abbreviations: CM = Cytosensor Microphysiometer; EPA = U.S. Environmental Protection Agency; EU = European Union; GHS = Globally Harmonized System; N = number of substances included in this analysis; No. = data used to calculate the percentage.

[1] GHS classification system (UN 2007): Category 1 vs. Category 2A/2B/NC.
EPA classification system (EPA 2003): Category I vs. Category II/III/IV.
EU classification system (EU 2001): R41 vs. R36/NL.

The ability of the CM test method to distinguish ocular corrosives and severe irritants (i.e., EPA Category I, EU R41, GHS Category 1) among the water-soluble nonsurfactant substances was evaluated for the EPA, EU, and GHS classification systems (EPA 2003; EU 2001; UN 2007).[11] As indicated in **Table 3-4**, overall accuracy ranged from 79% (23/29) to 92% (23/25) depending on the hazard classification system used. The false positive rate was 0% (0/17 or 0/18) for all classification systems used.

[10] The FHSA ocular hazard category that is assigned based on results from the Draize rabbit eye test does not distinguish between ocular corrosives/severe irritants and less severe irritants. For this reason, an evaluation of the CM test method as a screening test to identify ocular corrosives/severe irritants using the FHSA classification system is not possible.

[11] The FHSA ocular hazard category that is assigned based on results from the Draize rabbit eye test does not distinguish between ocular corrosives/severe irritants and less severe irritants. For this reason, an evaluation of the CM test method as a screening test to identify ocular corrosives/severe irritants using the FHSA classification system is not possible.

Table 3-4 Accuracy of the CM Test Method for Distinguishing Corrosives/Severe Irritants[1] from All Other Irritant Classes for Nonsurfactant Substances

	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
GHS	29	83	24/29	55	6/11	100	18/18	0	0/18	45	5/11
EPA	25	92	23/25	71	5/7	100	18/18	0	0/18	29	2/7
EU	29	79	23/29	50	6/12	100	17/17	0	0/17	50	6/12

Abbreviations: CM = Cytosensor Microphysiometer; EPA = U.S. Environmental Protection Agency; EU = European Union; GHS = Globally Harmonized System; N = number of substances included in this analysis; No. = data used to calculate the percentage.

[1] GHS classification system (UN 2007): Category 1 vs. Category 2A/2B/NC. EPA classification system (EPA 2003): Category I vs. Category II/III/IV. EU classification system (EU 2001): R41 vs. R36/NL.

3.2.4 Test Method Reliability

Interlaboratory Reproducibility

For the CM test method, intralaboratory reproducibility was assessed quantitatively based on calculated coefficients of variation (CVs) for MRD_{50} values for two different studies. Mean CVs ranged from 10% to 24% and tended to be slightly higher for surfactant substances than for nonsurfactant substances.

Interlaboratory reproducibility of the CM test method was also assessed using the data from the European Commission/Home Office (EC/HO; Balls et al. 1995) and European Cosmetic, Toiletry and Perfumery Association (COLIPA; Brantom et al. 1997) validation studies, which included four laboratories and two laboratories, respectively. Mean CVs in the EC/HO study ranged from 16% to 37% for surfactant substances and up to 51% for nonsurfactant substances. For surfactant materials, all four laboratories using the CM test method had 100% agreement for 55% (6/11) of the test substances; 75% of the laboratories had identical results for 27% (3/11) of the test substances; and 50% of the laboratories had agreement for 18% (2/11) of the test substances. For nonsurfactant substances, agreement among the laboratories was 100% for 48% (11/23) of the test substances, 75% for 22% (5/23) of the test substances, 67% for 4% (1/23) of the test substances, and 50% for 13% (3/23) of the test substances.

For the COLIPA study, substances were divided into surfactant materials, surfactant-based formulations and mixtures, and nonsurfactant substances. Two laboratories had mean between-laboratory CVs ranging from 16% to 23% for surfactant materials, approximately 16% for surfactant-based formulations and mixtures, and 32% to 51% for nonsurfactant substances. For surfactant materials, the laboratories had 100% agreement for 90% (9/10) of the test substances and 0% agreement for 10% (1/10) of them. The laboratories had 100% agreement for 100% (7/7) surfactant-based formulations and mixtures. For nonsurfactant substances, the laboratories had 100% agreement for 78% (7/9) of the test substances and 0% agreement for 22% (2/9) of them.

3.2.5 Animal Welfare Considerations

Except for the mice originally used to develop the L929 cell line, no animals are used for the CM test method.

4.0 The Hen's Egg Test–Chorioallantoic Membrane Test Method

The HET-CAM test method uses the chorioallantoic membrane, which is a vascular fetal membrane composed of the fused chorion and allantois. The acute effects induced by a test substance on the small blood vessels and proteins of this soft tissue membrane are used as an indicator of effects induced by the same test substance in the eye of a treated rabbit.

ICCVAM previously evaluated the validation status of the HET-CAM test method as an *in vitro* alternative to the Draize rabbit eye test to identify ocular corrosives and severe irritants (i.e., those that induce irreversible ocular damage; EPA Category I, EU R41, GHS Category 1) and determined that the reproducibility and accuracy was **not** sufficient to support its use for this purpose (ICCVAM 2006e). In the current evaluation, ICCVAM evaluated the validation status of the HET-CAM test as an *in vitro* alternative to the Draize rabbit eye test for identifying nonsevere ocular irritants (i.e., those that induce reversible ocular damage; EPA Category II and III, EU R36, GHS Category 2A and 2B) and substances not labeled as irritants (i.e., EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified) according to the EPA, EU, FHSA, and GHS classification systems (EPA 2003; EU 2001; FHSA 2005; UN 2007).

4.1 ICCVAM Recommendations

4.1.1 ICCVAM Recommendations: Test Method Usefulness and Limitations

Evaluation as a Screening Test to Identify Substances Not Labeled as Irritants
Based on the current evaluation, ICCVAM concludes that the scientific validity of the HET-CAM test method has been adequately evaluated and that the HET-CAM test method is **not** recommended as a screening test to distinguish substances not labeled as irritants (i.e., EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified) from all other hazard categories (i.e., EPA Category I, II, III; EU R41, R36; FHSA Irritant; GHS Category 1, 2A, 2B) when results are to be used specifically for hazard classification and labeling purposes under the EPA, EU, FHSA, or GHS hazard classification systems (EPA 2003; EU 2001; FHSA 2005; UN 2007). There are too few surfactants or oil/water emulsions in the moderate irritant categories to allow sufficient confidence in the ability of the HET-CAM test method to distinguish them from the substances not labeled as irritants (i.e., there were no GHS Category 2A substances and only two EPA Category II or EU R36 substances).

Identification of Reversible Eye Irritation Hazard Categories
ICCVAM further concludes that, based on an evaluation of available data and corresponding performance (accuracy and reliability), the HET-CAM test method is **not** recommended to identify moderate and mild ocular irritants as defined by the EPA, EU, and GHS classification systems (EPA 2003; EU 2001; UN 2007).[12]

Evaluation as a Screening Test to Identify Ocular Corrosives and Severe Irritants
The available validation database for the HET-CAM test method has remained unchanged since the original ICCVAM evaluation (ICCVAM 2006b). Therefore, the original ICCVAM recommendation for the use of the HET-CAM test method to identify substances as ocular corrosives and severe irritants remains unchanged:

> The use of the HET-CAM test method for screening and identifying ocular corrosives and severe irritants (i.e., EPA Category I, GHS Category 1, EU R41) in a tiered-testing strategy, as part of a weight-of-evidence approach, is **not** recommended (ICCVAM 2006e).

[12] The FHSA ocular hazard category that is assigned based on results from the Draize rabbit eye test does not distinguish between mild and moderate ocular irritants.

Independent Peer Review Panel Conclusions and Recommendations
The Panel concluded that the HET-CAM test method cannot identify substances from all hazard categories. The Panel also concluded (with one minority opinion) that the HET-CAM test method using the IS(A) analysis method cannot be used as a screening test to distinguish substances not labeled as irritants (i.e., EPA Category IV, EU Not Labeled, GHS Not Classified) from all other hazard categories (i.e., EPA Category I, II, III; EU R41, R36; GHS Category 1, 2A, 2B) when results are to be used for EPA, EU or GHS hazard classifications. The Panel reached this conclusion because there were too few surfactants or oil/water emulsions in the mild to moderate irritant categories to have sufficient confidence in the ability of the test to distinguish them from substances not labeled as irritants.

4.1.2 ICCVAM Recommendations: HET-CAM Test Method Protocol

The updated ICCVAM-recommended HET-CAM test method protocol is included as an appendix to this report (**Appendix B**). The protocol has been modified from a generic description of the IS analysis method to include a more detailed IS(A) analysis method to be used for prospective studies. However, a description of the IS(B) method is included for retrospective analyses, where IS(B) analysis method data could be converted to fixed time points similar to those used for the IS(A) analysis method. All future studies intended to further characterize the usefulness and limitations of the HET-CAM test method should be conducted using this protocol.

Independent Peer Review Panel Conclusions and Recommendations
The Panel concluded that the protocol is sufficiently detailed that it could be conducted reproducibly in other laboratories. However, they emphasized that the protocol should reflect any restrictions of the current applicability domain and it should also reflect details specific to the testing of certain types of substances. In addition, they noted that the protocol reflects the IS(A) analysis method, which was the subject of the ICCVAM draft recommendation, but additional data derived using the IS(B) analysis method could be collected and extrapolated to the IS(A) analysis method.

4.1.3 ICCVAM Recommendations: Future Studies for the HET-CAM Test Method

ICCVAM recommends that additional studies be conducted to further optimize the HET-CAM test method decision criteria that would be used to identify ocular corrosives and severe irritants (EPA Category I, EU R41, GHS Category 1), moderate and mild irritants (i.e., EPA Category II, III; EU R36; GHS Category 2A, 2B), and substances not labeled as irritants (i.e., EPA Category IV; EU Not Labeled; FHSA Not Labeled; GHS Not Classified) as defined by the EPA, EU, FHSA, and GHS classification systems. Such studies could potentially improve the usefulness of the HET-CAM test method for identifying these types of substances.

Additionally, in order to further optimize the protocol and more adequately characterize the usefulness of the HET-CAM test method for identifying substances not labeled as irritants:

- ICCVAM recommends that the applicability domain be expanded to include a broader range of chemical and product classes.
- ICCVAM encourages users to provide all data that are generated from future studies because they could be used to further characterize the usefulness and limitations of the HET-CAM test method for the identification of all ocular hazard categories.

Independent Peer Review Panel Conclusions and Recommendations
In order to further optimize the protocol and more adequately characterize the usefulness of the HET-CAM test method for identifying substances not labeled as irritants, the Panel recommended that additional data be collected on mild and moderate irritants and that the applicability domain be expanded to include a broader range of chemical and product classes.

They also noted that most of the single ingredients tested in the HET-CAM performed poorly, whereas formulations performed better. Hence, the effect of increasing the concentration of single ingredients on accuracy and sensitivity should be assessed in the HET-CAM to determine if there are test substance concentration limits for specific chemical classes.

The Panel did not support additional studies for using the HET-CAM test method to identify all categories of ocular irritants, given that it has been extensively evaluated and proven incapable for this task. However, as noted above, ICCVAM considers such studies valuable because they could improve the usefulness of the HET-CAM test method for identifying these types of substances. Furthermore, it is essential that the full range of ocular irritancy potential be tested in order to establish whether false negatives in the HET-CAM test method present a significant public health risk (e.g., EPA Category I substances predicted as Category IV in the HET-CAM test method).

4.1.4 ICCVAM Recommendations: Performance Standards for the HET-CAM Test Method

Based on the available data and associated performance described in the final ICCVAM BRD (**Appendix E**), ICCVAM recommends that the development of performance standards for the HET-CAM test method is not warranted at this time.

4.2 Validation Status of the HET-CAM Test Method

The following is a synopsis of the information in the final ICCVAM BRD (**Appendix E**), which reviews the available data and information for the HET-CAM test method. The ICCVAM BRD describes the current validation status of the HET-CAM test method, including what is known about its reliability and accuracy, the scope of the substances tested, and standardized protocols for the validation study.

4.2.1 Test Method Description

The HET-CAM protocol, first described by Luepke (1985), uses a vascular fetal membrane, the chorioallantoic membrane (CAM), which is composed of the fused chorion and allantois. The CAM has been proposed as a model for a living membrane (such as the conjunctiva) because it comprises a functional vasculature. Additionally, evaluation of coagulation (i.e., protein denaturation) may reflect corneal damage that may be produced by the test substance. The acute effects induced by a test substance on the small blood vessels and proteins of this soft tissue membrane are proposed to be similar to effects induced by the same test substance in the eye of a treated rabbit.

4.2.2 Validation Database

No new HET-CAM data were obtained since the ICCVAM evaluation of the HET-CAM test method for identifying ocular corrosives and severe irritants (ICCVAM 2006b). Therefore, the same database was used in the current evaluation. The database is composed of 260 substances representing a wide variety of chemical and product classes. It includes more than 50 commercial products or formulations. However, of the 260 substances, 167 could not be classified within a product class.

Analyses of each of the multiple HET-CAM protocols indicate that the IS(A) analysis method achieved the best performance when evaluating substances not labeled as irritants. The available IS(A) database includes a total of 63 test substances, 60 of which had sufficient *in vivo* data to be assigned an ocular irritancy hazard classification. Among these 60 substances are 43 cosmetic and personal care product formulations (including 25 surfactant-based formulations and 18 oil/water emulsions), and 17 individual substances (including seven alcohols; no other classes were represented by more than three substances).

Detailed *in vivo* data were necessary to calculate the appropriate EPA, EU, FHSA, and GHS ocular hazard classifications (EPA 2003; EU 2001; FHSA 2005; UN 2007) (**Appendix E**). These data

consist of cornea, iris and conjunctiva scores for each animal at 24, 48, and 72 hours and/or assessment of the presence or absence of lesions at 7, 14, and 21 days. Thus, some of the test substances for which there was only limited *in vivo* data could not be used to evaluate test method accuracy and reliability. In order to maximize the number of substances included in these analyses, "proportionality" criteria (i.e., FHSA-20% and FHSA-67%) were applied for the purpose of assigning an FHSA classification for test results that would require additional testing according to the FHSA sequential testing strategy (see **Section 2.2.2**).

4.2.3 Test Method Accuracy

Identification of All Ocular Hazard Categories

The ability of the HET-CAM test method to identify all categories of ocular irritation potential was evaluated for the EPA, EU, and GHS classification systems (EPA 2003; EU 2001; UN 2007).[13] As indicated in **Table 4-1**, overall correct classifications ranged from 38% (23/60) to 41% (24/59) depending on the classification system used when evaluating the entire database.

It is apparent from **Table 4-1** that the limited number of substances (n = 0–2) in the moderate irritant category (i.e., EPA Category II, EU R36, and GHS Category 2A) prevents an adequate evaluation of HET-CAM performance for this category. Similarly, while there are 18 substances classified as EPA Category III, there are only five substances classified as GHS Category 2B (the EU system does not distinguish mild irritants).

Distinguishing Substances Not Labeled as Irritants from All Other Hazard Categories

The ability of the HET-CAM test method to distinguish substances not labeled as irritants (i.e., EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified) from all other ocular hazard categories (i.e., EPA Category I, II, III; EU R41, R36; FHSA Irritant; GHS Category 1, 2A, 2B) as defined by the EPA, EU, FHSA, and GHS classification systems (EPA 2003; EU 2001; FHSA 2005; UN 2007) was also evaluated.

As indicated in **Table 4-2**, overall accuracy for the identification of substances not labeled as irritants (i.e., EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified) from all other categories ranged from 62% (36/58) to 80% (44/55) depending on the hazard classification system used. False positive and false negative rates ranged from 60% (9/15) to 69% (22/32) and 0% (0/26 or 0/36) to 9% (4/45 or 4/47), respectively. Among the four false negatives for the EPA and FHSA-20% systems, 100% (4/4, all oil/water emulsion cosmetic formulations) were EPA Category III substances based on a conjunctival redness score of 2 that required at least 3 days to resolve. For one of these substances, one of the six rabbits tested had a conjunctival redness score of 2 that required 14 days to resolve. Four of the remaining five rabbits in this study had conjunctival redness scores of 2 that resolved within three days. The fifth rabbit did not have this lesion.

[13] The FHSA ocular hazard category that is assigned based on results from the Draize rabbit eye test does not distinguish between ocular corrosives/severe irritants and less severe irritants. For this reason, an evaluation of the HET-CAM test method to identify all ocular hazard categories using the FHSA classification system is not possible.

Table 4-1 Evaluation of the Performance of the HET-CAM Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the EPA, EU, and GHS Classification Systems[1]

	Overall Correct Classification	Severe[2]		Moderate[3]			Mild[4]			Not Labeled[5]	
		Actual	Under	Over	Actual	Under	Over	Actual	Under	Over	Actual
EPA	38% (23/60)	48% (12/25)	52% (13/25)	50% (1/2)	50% (1/2)	0% (0/2)	56% (10/18)	22% (4/18)	22% (4/18)	60% (9/15)	40% (6/15)
GHS	41% (24/59)	50% (13/26)	50% (13/26)	0% (0/0)	0% (0/0)	0% (0/0)	80% (4/5)	20% (1/5)	0% (0/5)	64% (18/28)	36% (10/28)
EU	40% (23/58)	50% (12/24)	50% (12/24)	50% (1/2)	50% (1/2)	0% (0/2)	NA	NA	NA	69% (22/32)	31% (10/32)

Abbreviations: EPA = U.S. Environmental Protection Agency; EU = European Union; GHS = Globally Harmonized System; HET-CAM = hen's egg test–chorioallantoic membrane; NA = not applicable.

[1] EPA classification system (EPA 2003); EU classification system (EU 2001); GHS classification system (UN 2007).

[2] Severe = GHS Category 1; EPA Category I; EU R41.

[3] Moderate = GHS Category 2A; EPA Category II; EU R36.

[4] Mild = GHS Category 2B; EPA Category III.

[5] Not Labeled = GHS Not Classified; EPA Category IV; EU Not Labeled.

Table 4-2 Accuracy of the HET-CAM Test Method for Distinguishing Substances Not Labeled as Irritants[1] from All Other Irritant Classes

	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
EPA	60	78	47/60	91	41/45	40	6/15	60	9/15	9	4/45
GHS	59	69	41/59	100	31/31	36	10/28	64	18/28	0	0/31
EU	58	62	36/58	100	26/26	31	10/32	69	22/32	0	0/26
FHSA-20%	63	78	49/63	91	43/47	38	6/16	63	10/16	9	4/47
FHSA-67%	55	80	44/55	97	38/39	38	6/16	63	10/16	3	1/39

Abbreviations: EPA = U.S. Environmental Protection Agency; EU = European Union; FHSA = U.S. Federal Hazardous Substances Act; GHS = Globally Harmonized System; HET-CAM = HET-CAM = hen's egg test–chorioallantoic membrane; N = number of substances included in this analysis; No. = data used to calculate the percentage.

[1] GHS classification system (UN 2007): Not Classified vs. Category 1/2A/2B.
EPA classification system (EPA 2003): Category IV vs. Category I/II/III.
EU classification system (EU 2001): Not Labeled vs. R41/R36.
FHSA classification system (FHSA 2005): Not Labeled vs. Irritant.

4.2.4 Test Method Reliability

Interlaboratory Reproducibility

Quantitative and qualitative evaluations of HET-CAM test method reliability have been conducted previously (ICCVAM 2006b). Because the database used for the current evaluation of the HET-CAM test method has not changed, the quantitative evaluation of test method reliability remains unchanged. However, additional qualitative analyses of interlaboratory reproducibility were conducted to evaluate the extent of agreement of HET-CAM hazard classifications among the five laboratories participating in the interlaboratory validation study (Hagino et al. 1999). As was done for the accuracy evaluation, these qualitative evaluations of reproducibility were based on (1) the use of the HET-CAM test method for identifying all ocular hazard categories according to the EPA, EU, and GHS systems, and (2) the use of the HET-CAM test method to distinguish substances not labeled as irritants (i.e., EPA Category IV, EU Not Labeled, GHS Not Classified) from all other irritant categories (i.e., EPA Category I, II, III; EU R41, R36; GHS Category 1, 2A, 2B). Given that the performance of the HET-CAM test method was similar for the EPA and FHSA classification systems, additional reliability analyses were not conducted for the FHSA classification system.

Using the first approach (i.e., identifying all ocular hazard categories), there was 100% agreement among the five laboratories for a majority of the Draize ocular corrosives and severe irritants correctly classified by the HET-CAM test method based on all three classification systems. (There was 100% agreement for 63% [5/8] of the correctly identified EPA Category I substances and 100% agreement for 71% [5/7] of the correctly identified GHS Category 1 or EU R41 substances.) There was 100% agreement among the five laboratories for the one moderate irritant in the database (EPA Category II or EU R36; no GHS Category 2A substances were included), which was overpredicted. There was 100% agreement for the mild ocular irritants (i.e., EPA Category III, GHS Category 2B; the EU does not have a mild irritant category), which were uniformly overpredicted. For the Hagino et al. (1999) database, all of the substances not classified as irritants based on Draize results (i.e., EPA Category IV, EU Not Labeled, GHS Not Classified) were overclassified by the HET-CAM test method. There was 100% agreement among the five laboratories for 86% (6/7) or 75% (3/4) of these substances for the EU and GHS classification systems, respectively. By comparison, for the two EPA Category IV substances tested, there was either 100% or 80% agreement among the five laboratories.

Using the second approach (i.e., identifying substances not labeled as irritants), there was 100% agreement among the five laboratories for 82% (14/17), 76% (13/17), and 94% (16/17) for the 17 substances included in the Hagino et al. (1999) database for the EPA, EU, and GHS classification systems, respectively.

There was 100% agreement among the five laboratories for 100% (13/13) of the substances correctly identified as irritants according to the EPA classification system (i.e., Category I, II, or III). While neither of the EPA Category IV substances was correctly identified by the HET-CAM test method, there was 60% agreement among the five laboratories for 100% (2/2) of the EPA Category IV substances that were overpredicted by the HET-CAM test method.

There was 100% agreement among the five laboratories for 63% (5/8) of the substances correctly identified as an irritant according to the EU classification system (i.e., R36 or R41). There was at least 60% agreement among the five laboratories for the remaining three substances correctly classified as an irritant. While none of the EU Not Labeled substances were correctly identified by the HET-CAM test method, there was 100% agreement among the five laboratories for 86% (6/7) of these substances that were overpredicted by the HET-CAM test method.

There was 100% agreement among the five laboratories for 100% (11/11) of the substances correctly identified as irritants according to the GHS classification system (i.e., Category 1, 2A, or 2B). While none of the GHS Not Classified substances was correctly identified by the HET-CAM test method, there was 100% agreement among the five laboratories for 75% (3/4) of these substances that were overpredicted by the HET-CAM test method.

As stated above, this review provides a comprehensive summary of the current validation status of the HET-CAM test method, including what is known about its reliability and accuracy, and the scope of the substances tested. Raw data for the HET-CAM test method will be maintained for future use, so that these performance statistics may be updated as additional information becomes available.

4.2.5 Animal Welfare Considerations

The HET-CAM test method has the potential to reduce and refine animal use in eye irritation testing. It would refine animal use by the *in vitro* identification of ocular corrosives/severe irritants, nonsevere irritants, or substances not labeled as irritants in a tiered-testing strategy.

5.0 The Isolated Chicken Eye Test Method

The ICE test method is an *in vitro* eye irritation test method using chicken eyes that are byproducts from processing plants. In the ICE test method, damage by the test substance is assessed by determining corneal swelling, opacity, and fluorescein retention. These endpoints are used collectively as an indicator of effects induced by the test substance in the eye of a treated rabbit.

ICCVAM previously evaluated the validation status of the ICE test method as an *in vitro* alternative to the Draize rabbit eye test to identify ocular corrosives/severe irritants (i.e., those that induce irreversible ocular damage; EPA Category I, EU R41, GHS Category 1) and determined that the reproducibility and accuracy was sufficient to support its use for this purpose for some types of substances (ICCVAM 2006e). U.S. agencies and international organizations (OECD 2009b) have adopted the ICE test method for this purpose. Following this initial evaluation, ICCVAM evaluated the validation status of the ICE test method as an *in vitro* alternative to the Draize rabbit eye test for identifying nonsevere ocular irritants (i.e., those that induce reversible ocular damage [EPA Category II and III, EU R36, GHS Category 2A and 2B]) and substances not labeled as irritants (i.e., EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified) according to the EPA, EU, FHSA, and GHS classification systems (EPA 2003; EU 2001; FHSA 2005; UN 2007).

5.1 ICCVAM Recommendations

5.1.1 ICCVAM Recommendations: Test Method Usefulness and Limitations

Evaluation as a Screening Test to Identify Substances Not Labeled as Irritants
ICCVAM concludes that the ICE test method is **not** recommended as a screening test to distinguish substances not labeled as irritants (i.e., EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified) from all other hazard categories (i.e., EPA Category I, II, III; EU R41, R36; FHSA Irritant; GHS Category 1, 2A, 2B) when results are to be used specifically for hazard classification and labeling purposes under the EPA, EU, FHSA, and GHS classification systems (EPA 2003; EU 2001; FHSA 2005; UN 2007).

Identification of Reversible Eye Irritation Hazard Categories
Based on an evaluation of available data and test method performance (accuracy and reliability), ICCVAM concludes that the ICE test method is **not** recommended to identify moderate and mild ocular irritants as defined by the EPA, EU, and GHS classification systems (EPA 2003; EU 2001; UN 2007).[14]

Evaluation as a Screening Test to Identify Ocular Corrosives and Severe Irritants
The available validation database for the ICE test method has not changed since the original ICCVAM evaluation (ICCVAM 2006c). Therefore, the original ICCVAM recommendation for the use of the ICE test method to identify substances as ocular corrosives and severe irritants remains unchanged:

> There are sufficient data to support the use of the ICE test method, in appropriate circumstances and with certain limitations, as a screening test to identify substances as ocular corrosives and severe irritants (i.e., EPA Category I, UN GHS Category 1, EU R41) in a tiered-testing strategy, as part of a weight-of-evidence approach. In a tiered-testing strategy, when a positive result is obtained in an appropriately validated *in vitro* test, a test substance may be classified as an ocular hazard without testing in rabbits. A substance that tests negative in the *in vitro* ocular toxicity test would need to be tested in the *in vivo* ocular test to identify possible *in vitro* false negatives and to identify moderate and mild ocular irritants (ICCVAM 2006e).

[14] The FHSA ocular hazard category that is assigned based on results from the Draize rabbit eye test does not distinguish between mild and moderate ocular irritants.

Independent Peer Review Panel Conclusions and Recommendations
The Panel concluded that the available data and test method performance (accuracy and reliability) supported the ICCVAM draft recommendation that the ICE test method is not recommended to identify substances from all hazard categories as defined by the EPA, EU, and GHS classification systems. The Panel further concluded that the ICE test method is not recommended as a screening test to distinguish substances as not labeled as irritants from all other hazard categories as defined by the EPA, EU, and GHS classification systems.

5.1.2 ICCVAM Recommendations: ICE Test Method Protocol

For use of the ICE test method as a screening test to identify substances as ocular corrosives and severe irritants (i.e., EPA Category I, EU R41, GHS Category 1), ICCVAM recommends using the updated ICCVAM ICE test method protocol that is included as an appendix to this report (**Appendix B**). In addition, all future studies intended to further characterize the usefulness and limitations of the ICE test method should be conducted using this protocol.

Independent Peer Review Panel Conclusions and Recommendations
The Panel concluded that the protocol is sufficiently detailed but noted that the protocol could be improved by adding objective endpoints for corneal opacity and fluorescein staining.

5.1.3 ICCVAM Recommendations: Future Studies for the ICE Test Method

To further the use of this test method and to evaluate its use as a potential replacement for the Draize rabbit eye test or for the identification of mild and moderate ocular irritants (i.e., EPA Category II, III; EU R36; GHS Category 2A, 2B) and substances not labeled as irritants (i.e., EPA Category IV; EU Not Labeled; FHSA Not Labeled; GHS Not Classified), ICCVAM recommends additional studies be considered and undertaken.

- Additional optimization studies/evaluations should be conducted in an attempt to improve the correct classification of mild and moderate ocular irritants and substances not labeled as irritants. After optimization, additional studies to further assess the reliability and accuracy of the test method are recommended.
- ICCVAM recommends that a histopathological evaluation of the corneal tissue, using standardized procedures, be included when the ICE test method is conducted. Such data will allow for development of decision criteria and future assessments on the usefulness of this endpoint for classifying and labeling substances, especially those that may otherwise produce borderline or false negative results.
- ICCVAM encourages users to provide all data that are generated from future studies, as they could be used to further characterize the usefulness and limitations of the ICE test method for the identification of all ocular hazard categories.

Independent Peer Review Panel Conclusions and Recommendations
The Panel concluded that additional optimization studies should be required to validate the test method for the identification of all ocular irritation hazard categories. The Panel also noted that the use of histopathology to evaluate corneal tissue might improve test method accuracy.

5.1.4 ICCVAM Recommendations: Performance Standards for the ICE Test Method

Based on the available data and associated performance described in the final ICCVAM BRD (**Appendix F**), ICCVAM recommends that the development of performance standards for the ICE test method is not warranted at this time.

5.2 Validation Status of the ICE Test Method

The following is a synopsis of the information in the final ICCVAM BRD (**Appendix F**), which reviews the available data and information for the ICE test method. The ICCVAM BRD describes the

current validation status of the ICE test method, including what is known about its reliability and accuracy, the scope of the substances tested, and standardized protocols for the validation study.

5.2.1 Test Method Description

The ICE test method is an *in vitro* model that provides short-term maintenance of the chicken eye. In the ICE test method, damage by the test substance is assessed by determination of corneal swelling, opacity, and fluorescein retention. While the latter two parameters involve a qualitative assessment, analysis of corneal swelling provides for a quantitative assessment. Each measurement is either converted into a quantitative score used to calculate an overall irritation index, or assigned a qualitative categorization that is used to assign an *in vitro* ocular irritation classification. Either of these outcomes can then be used to predict the *in vivo* ocular irritation potential of a test substance.

5.2.2 Validation Database

No new ICE data were obtained after the ICCVAM evaluation of the ICE test method for identifying ocular corrosives and severe irritants (ICCVAM 2006c). Therefore, the same database was used in the current evaluation. It is composed of 175 substances representing a wide variety of chemical and product classes. However, of the 175 substances, 85 (including formulations of unidentified composition) could not be assigned a specific chemical class.

Detailed *in vivo* data were necessary to calculate the appropriate EPA, EU, FHSA, and GHS ocular hazard classifications (EPA 2003; EU 2001; FHSA 2005; UN 2007) (**Appendix F**). These data consist of cornea, iris and conjunctiva scores for each animal at 24, 48, and 72 hours and/or assessment of the presence or absence of lesions at 7, 14, and 21 days. Thus, some of the test substances for which there was only limited *in vivo* data could not be used to evaluate test method accuracy and reliability. In order to maximize the number of substances included in these analyses, "proportionality" criteria (i.e., FHSA-20% and FHSA-67%) were applied for the purpose of assigning an FHSA classification for test results that would require additional testing according to the FHSA sequential testing strategy (see **Section 2.2.2**).

5.2.3 Test Method Accuracy

Identification of All Ocular Hazard Categories

The ability of the ICE test method to identify all categories of ocular irritation potential was evaluated for the EPA, EU, and GHS classification systems (EPA 2003; EU 2001; UN 2007).[15] As indicated in **Table 5-1**, overall correct classifications ranged from 59% (83/141) to 77% (118/153) depending on the classification system used when evaluating the entire database.

[15] The FHSA ocular hazard category that is assigned based on results from the Draize rabbit eye test does not distinguish between ocular corrosives/severe irritants and less severe irritants. For this reason, an avaluation of the ICE test method to identify all ocular hazard categories using the FHSA classification system is not possible.

Table 5-1 Evaluation of the Performance of the ICE Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the EPA, EU, and GHS Classification Systems[1]

	Overall Correct Classification	Severe[2]		Moderate[3]			Mild[4]			Not Labeled[5]	
		Actual	Under	Over	Actual	Under	Over	Actual	Under	Over	Actual
EPA	62% (87/140)	48% (13/27)	52% (14/27)	31% (5/16)	50% (8/16)	19% (3/16)	29% (11/38)	53% (20/38)	18% (7/38)	22% (13/59)	78% (46/59)
EU	77% (118/153)	59% (19/32)	41% (13/32)	18% (5/28)	57% (16/28)	25% (7/28)	NA	NA	NA	11% (10/93)	89% (83/93)
GHS	59% (83/141)	52% (15/29)	48% (14/29)	36% (8/22)	36% (8/22)	28% (6/22)	18% (2/11)	73% (8/11)	9% (1/11)	34% (27/79)	66% (52/79)

Abbreviations: EPA = U.S. Environmental Protection Agency; EU = European Union; GHS = Globally Harmonized System; ICE = isolated chicken eye; NA = not applicable.

[1] EPA classification system (EPA 2003); GHS classification system (UN 2007); EU classification system (EU 2001).

[2] Severe = EPA Category I; GHS Category 1; EU R41.

[3] Moderate = EPA Category II; GHS Category 2A; EU R36.

[4] Mild = EPA Category III; GHS Category 2B.

[5] Not Labeled = EPA Category IV; EU Not Labeled; GHS Not Classified.

Distinguishing Substances Not Labeled as Irritants from All Other Hazard Categories
The ability of the ICE test method to distinguish substances not labeled as irritants (i.e., EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified) from all other ocular hazard categories (i.e., EPA Category I, II, III; EU R41, R36; FHSA Irritant; GHS Category 1, 2A, 2B) as defined by the EPA, EU, FHSA, and GHS classification systems (EPA 2003; EU 2001; FHSA 2005; UN 2007) was also evaluated.

As indicated in **Table 5-2**, overall accuracy for the identification of substances not labeled as irritants (i.e., EPA Category IV, EU Not Labeled, FHSA Not Labeled, GHS Not Classified) from all other categories ranged from 78% (110/141) to 85% (130/153) depending on the hazard classification system used. The false negative rates ranged from 6% (4/62) to 22% (13/60) depending on the hazard classification system used. The lowest false negative rate (6%) was noted for the GHS system, followed by 9% (7/76) for the FHSA-67% system, 12% (10/84) for the FHSA-20% system, 14% (11/81) for the EPA system, and 22% (13/60) for the EU system. However, at least one of these false negatives is classified as an ocular corrosive and severe irritant based on Draize rabbit eye test data (n = 1 each for the EPA and GHS systems and n = 6 for the EU system).

Table 5-2 Accuracy of the ICE Test Method for Distinguishing Substances Not Labeled as Irritants[1] from All Other Irritant Classes

	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
EPA	140	83	116/140	86	70/81	78	46/59	22	13/59	14	11/81
GHS	141	78	110/141	94	58/62	66	52/79	34	27/79	6	4/62
EU	153	85	130/153	78	47/60	89	83/93	11	10/93	22	13/60
FHSA-20%	146	83	121/146	88	74/84	76	47/62	24	15/62	12	10/84
FHSA-67%	138	84	116/138	91	69/76	76	47/62	24	15/62	9	7/76

Abbreviations: EPA = U.S. Environmental Protection Agency; EU = European Union; FHSA = U.S. Federal Hazardous Substances Act; GHS = Globally Harmonized System; ICE = isolated chicken eye; N = number of substances included in this analysis; No. = data used to calculate the percentage.

[1] GHS classification system (UN 2007): Not Classified vs. Category 1/2A/2B.
EPA classification system (EPA 2003): Category IV vs. Category I/II/III.
EU classification system (EU 2001): Not Labeled vs. R41/R36.
FHSA classification system (FHSA 2005): Not Labeled vs. Irritant.

5.2.4 Test Method Reliability

Interlaboratory Reproducibility
Quantitative and qualitative evaluations of ICE test method reliability have been conducted previously (ICCVAM 2006c). However, additional qualitative analyses of interlaboratory reproducibility were conducted to evaluate the extent of agreement of ICE hazard classifications among the four laboratories participating in the interlaboratory validation study (Balls et al. 1995). As was done for the accuracy evaluation, these qualitative evaluations of reproducibility were based on (1) the use of the ICE test method for identifying all ocular hazard categories according to the EPA, EU, or GHS systems, and (2) the use of the ICE test method to distinguish substances not labeled as irritants (i.e., EPA Category IV, EU Not Labeled, GHS Not Classified) from all other ocular hazard categories (i.e., EPA Category I, II, or III; EU R41 or R36; GHS Category 1, 2A, or 2B). Given that the performance of the ICE test method was similar for the EPA and FHSA classification systems, additional reliability analyses were not conducted for the FHSA classification system.

Using the first approach (i.e., identifying all ocular hazard categories), there was 100% agreement among the four laboratories for a majority of the Draize ocular corrosives and severe irritants for all three classification systems, whether the substances were correctly identified or underclassified by the ICE test method. (For example, for the EPA system, there was 100% agreement for 70% [7/10] of the correctly identified Category I substances.) There was 100% agreement among the four laboratories for at least 50% (3/6 to 3/5) of the correctly identified moderate ocular irritants (EPA Category II, GHS Category 2A, EU R36). For the mild ocular irritants (EPA Category III, GHS Category 2B), there was 100% agreement among the four laboratories for 0% (0/2) to 13% (1/8) of the correctly identified substances. The four laboratories had only 50% agreement for 50% (4/8 or 1/2) of these substances for the EPA and GHS classification systems.

Among the four laboratories, a majority of the substances not classified as irritants based on Draize results (i.e., EPA Category IV, EU Not Labeled, GHS Not Classified) were overclassified by the ICE test method. The four laboratories had at least 75% agreement for all but two of these substances. For example, there was at least 75% agreement for 85% (11/13) of the GHS Not Labeled substances overclassified by the ICE test method. The four laboratories had at least 75% agreement for 76% (13/17) of the EU Not Labeled substances, whether they were correctly identified or overclassified by the ICE test method. For example, there was at least 75% agreement for 77% (7/9) of the EU Not Labeled substances that were correctly identified and 75% (6/8) of those overclassified by the ICE test method.

Using the second approach (i.e., distinguishing substances not labeled as irritants from all other ocular hazard categories), there was 100% agreement among the four laboratories for 61% (36/59) to 75% (44/59) of the substances included in the Balls et al. (1995) study.

There was 100% agreement among the four laboratories for 81% (38/47) of the substances correctly identified as irritants according to the EPA system (i.e., Category I, II, or III). While none of the EPA Category IV substances was correctly identified by the ICE test method, there was 75% agreement among the four laboratories for both of the Category IV substances that were overpredicted by the ICE test method.

The four laboratories had 100% agreement for 87% (33/38) of the substances correctly identified as irritants according to the GHS system (i.e., Category 1, 2A, or 2B). While only one of the GHS substances not labeled as irritants was correctly identified by the ICE test method (for which there was 75% agreement among the laboratories), there was at least 75% agreement among the four laboratories for 85% (11/13) of the GHS substances not labeled as irritants that were overpredicted by the ICE test method.

There was 100% agreement among the four laboratories for 85% (22/26) of the substances correctly identified as irritants according to the EU system (i.e., R36 or R41). The laboratories had at least 75% agreement for 77% (7/9) of the substances correctly identified as Not Labeled.

The final ICCVAM BRD (**Appendix F**) provides a comprehensive summary of the current validation status of the ICE test method, including what is known about its reliability and accuracy, and the scope of the substances tested. Raw data for the ICE test method will be maintained for future use, so that these performance statistics may be updated as additional information becomes available.

5.2.5 Animal Welfare Considerations

The ICE test method refines animal use. Because these animals are being humanely processed for nonlaboratory purposes, the testing procedure inflicts no additional pain or distress on animals. Substances that are identified as corrosive or severe irritants *in vitro* are excluded from *in vivo* testing.

The ICE test method can also reduce animal use. The test method utilizes animal species routinely raised as a food source in large numbers to replace the need for laboratory animals.

6.0 The Isolated Rabbit Eye Test Method

The IRE test method is an *in vitro* eye irritation test method using eyes from rabbits that have been euthanized for other research purposes or are byproducts from processing plants. In the IRE test method, the treated eye may be evaluated for corneal opacity, corneal swelling, fluorescein penetration, and effects on the corneal epithelium at various times over a four-hour observation period.

ICCVAM previously evaluated the validation status of the IRE test method as an *in vitro* alternative to the Draize rabbit eye test to identify ocular corrosives and severe irritants (i.e., those that induce irreversible ocular damage; EPA Category I, EU R41, GHS Category 1) and determined that the reproducibility and accuracy was **not** sufficient to support its use for this purpose (ICCVAM 2006e). In the current evaluation, ICCVAM evaluated the validation status of the IRE test method as an *in vitro* alternative to the Draize rabbit eye test for identifying nonsevere ocular irritants (i.e., those that induce reversible ocular damage [EPA Category II and III; EU R36; GHS Category 2A and 2B]) and substances not labeled as irritants (i.e., EPA Category IV; EU Not Labeled; FHSA Not Labeled; GHS Not Classified) according to the EPA, EU, FHSA, and GHS classification systems (EPA 2003; EU 2001; FHSA 2005; UN 2007).

6.1 ICCVAM Recommendations

6.1.1 ICCVAM Recommendations: Test Method Usefulness and Limitations

Evaluation as a Screening Test to Identify All Ocular Hazard Categories
There are insufficient data using all four recommended IRE endpoints (i.e., corneal opacity, corneal swelling, fluorescein penetration, and effects on the corneal epithelium) in a single study to assess test method accuracy and reliability. Among the studies that included all four recommended IRE endpoints, decision criteria are focused on distinguishing ocular corrosives and severe irritants from all other ocular hazard categories (i.e., moderate and mild irritants and substances not labeled as irritants) and do not specify decision criteria for each ocular hazard category. For these reasons, an adequate evaluation of the IRE test method for its ability to identify all ocular hazard categories is not feasible at this time.

Evaluation as a Screening Test to Identify Ocular Corrosives and Severe Irritants
The available validation database for the IRE test method has not changed since the original ICCVAM evaluation (ICCVAM 2006d). Therefore, the original ICCVAM recommendation for the use of the IRE test method to identify substances as ocular corrosives and severe irritants remains unchanged:

> The use of the IRE test method for screening and identifying ocular corrosives and severe irritants (i.e., EPA Category I, GHS Category 1, EU R41) in a tiered-testing strategy, as part of a weight-of-evidence approach, is **not** recommended (ICCVAM 2006e).

Independent Peer Review Panel Conclusions and Recommendations
The Panel concluded that there are insufficient data from all four recommended IRE test method endpoints (i.e., corneal opacity, fluorescein penetration, corneal swelling, and effects on the corneal epithelium) to evaluate the accuracy and reliability of the test method when all four endpoints are evaluated in a single study. Therefore, the Panel recommended that additional optimization and validation studies be conducted to further evaluate the relevance and reliability of the IRE test method, and in turn develop more definitive recommendations.

6.1.2 ICCVAM Recommendations: IRE Test Method Protocol

An ICCVAM-recommended test method protocol for the IRE test method that should be used for all future studies is included as an appendix to this report (**Appendix B**). The recommended protocol

remains unchanged from the previous ICCVAM evaluation (ICCVAM 2006e) and includes all four recommended IRE endpoints that should be measured: maximal corneal opacity (opacity × area), maximal corneal swelling, fluorescein penetration (intensity × area) and assessment of epithelial integrity (0.5, 1, 2, 3, and 4 hours).

Independent Peer Review Panel Conclusions and Recommendations
The Panel recommended that there should be rigid criteria specifying the handling and storage of the eyes. The Panel emphasized the need for control of the length of time between death and study initiation to account for any postmortem effects on the eye and criteria for appropriate inclusion/exclusion of ocular tissue. The Panel further emphasized the importance of criteria on test article administration/washout (e.g., viscous substances).

6.1.3 ICCVAM Recommendations: Future Studies for the IRE Test Method

To further the use of this test method and to evaluate the use of the IRE test method as a potential replacement for the Draize rabbit eye test or for the identification of all ocular hazard categories (i.e., EPA Category I, II, III, IV; GHS Category 1, 2A, 2B, Not Classified; FHSA Irritant, Not Labeled; EU R41, R36, Not Labeled), ICCVAM recommends additional studies be considered and undertaken.

- Additional evaluation studies should be conducted to increase the current IRE database and optimize the IRE test method decision criteria. Once these studies are conducted, ICCVAM recommends that additional validation studies be conducted to further evaluate the relevance and reliability of the IRE test method.
- ICCVAM recommends that a histopathological evaluation of the corneal tissue, using standardized procedures, be included when the IRE test method is conducted. Such data will allow for development of decision criteria and future assessments on the usefulness of this endpoint for classifying and labeling substances, especially those that may otherwise produce borderline or false negative results.
- ICCVAM encourages users to provide all data that are generated from future studies, as they could be used to further characterize the usefulness and limitations of the IRE test method for the identification of all ocular hazard categories.

Independent Peer Review Panel Conclusions and Recommendations
The Panel recommended a validation study to compare the utility of shipped rabbit eyes versus freshly collected rabbit eyes. Specifically, the Panel recommended inclusion of the study of shipping effects on ocular tissues into the planned validation study by GlaxoSmithKline and SafePharm. The Panel reiterated its concerns that there should be rigid criteria on the handling and storage of the eyes. In addition, the Panel recommended development of appropriate inclusion/exclusion criteria for eyes. Finally, the Panel recommended that criteria on test article administration/washout (e.g., viscous substances) should be developed.

6.1.4 ICCVAM Recommendations: Performance Standards for the IRE Test Method

Given that there are insufficient data using all four recommended IRE endpoints (i.e., corneal opacity, corneal swelling, fluorescein penetration, and effects on the corneal epithelium) in a single study to assess test method accuracy and reliability, ICCVAM recommends that the development of performance standards for the IRE test method is not warranted at this time.

6.2 Validation Status of the IRE Test Method

The IRE BRD (ICCVAM 2006d) describes the current validation status of the IRE test method, including what is known about its reliability and accuracy, the scope of the substances tested, and standardized protocols for the validation study.

6.2.1 Test Method Description

The IRE test method was developed by Burton and his colleagues at Unilever Research Laboratory, Colworth, United Kingdom, as an *in vitro* alternative to the Draize rabbit eye test for the assessment of eye irritation (Burton et al. 1981). In the IRE test method, liquid test substances are spread using a syringe, and solids are pulverized and applied as a powder over the corneas of enucleated rabbit eyes. The principal advantages of this test method are that the animals are euthanized prior to ocular irritancy testing (i.e., eyes from animals used for other toxicological purposes or from the food chain can be used) and testing is performed on the cornea, which is the part of the eye that is generally given the highest weight for scoring ocular irritancy in the Draize rabbit eye test. The effects of the test substance on the cornea of the isolated eye are measured quantitatively as an increase in thickness (swelling); subjectively as scores for corneal opacity, the area of corneal involvement, and fluorescein penetration; and descriptively as morphological changes to the corneal epithelium. However, the number of ocular parameters and the number of time points measured varies from study to study.

Two additional refinements of the IRE test method may be incorporated into the protocol or used *ad hoc* to supplement existing data. One is histopathological evaluation to confirm or identify the extent of irritancy at the cellular level, especially when the degree of irritancy falls between moderate and severe. Another is confocal microscopy to determine the extent and depth of ocular injury (Maurer et al. 2002). Many studies using the IRE test method evaluate single or multiple ocular endpoints at various times and then assign irritancy classifications to the substances tested (CEC 1991; Köeter and Prinsen 1985; Cooper et al. 2001; Jones et al. 2001), while others use mean data from one or more ocular endpoints assessed at various times after application of the test substance, typically 0.5 to 4 hours (Balls et al. 1995; Gettings et al. 1996). One protocol for the IRE test method was designed to specifically identify severe eye irritants (Guerriero et al. 2004). In this study, cut-off values for each ocular parameter tested were predetermined. If these cut-off values were achieved or exceeded in any single parameter over a period of 0.5 to 4 hours, including a significant change in the corneal epithelium, the test substance was classified as a severe eye irritant with potential to cause serious or irreversible damage to the human eye.

6.2.2 Validation Database

The available validation database for the IRE test method has remained unchanged since the original ICCVAM evaluation (ICCVAM 2006d). A total of 149 substances were evaluated in five studies, of which 25 were commercial products or formulations (ICCVAM 2006d). The chemical classes tested included but were not limited to alcohols, amides, amines, carboxylic acids, esters, ethers, formulations, heterocyclic, ketones, onium compounds, and sulfur compounds. The commercial products or formulations tested were skin cleansers, soaps, shampoos, conditioners, surfactants, and solvents.

Detailed *in vivo* data consisting of cornea, iris, and conjunctiva scores for each animal at 24, 48, and 72 hours and/or assessment of the presence or absence of lesions at 7, 14, and 21 days were necessary to calculate the appropriate EPA, EU, and GHS ocular hazard classifications (EPA 2003; EU 2001; UN 2007) (ICCVAM 2006d). Thus, some of the test substances for which there were only limited *in vivo* data could not be used to evaluate test method accuracy and reliability.

6.2.3 Test Method Accuracy

There are insufficient data using all four recommended IRE endpoints (i.e., corneal opacity, corneal swelling, fluorescein penetration, and effects on the corneal epithelium) in a single study to assess test method accuracy and reliability. Among the studies that included all four recommended IRE endpoints, decision criteria are focused on distinguishing ocular corrosives and severe irritants from all other ocular hazard categories (i.e., moderate and mild irritants and substances not labeled as irritants) and do not specify decision criteria for each ocular hazard category. For these reasons, an

adequate evaluation of the IRE test method for its ability to identify all ocular hazard categories is not feasible at this time.

6.2.4 Test Method Reliability

Due to the lack of quantitative IRE test method data for replicate experiments within an individual laboratory, an evaluation of the intralaboratory repeatability and reproducibility of the IRE test method could not be conducted. However, multilaboratory qualitative and quantitative IRE test method data were available for a collaborative study by the CEC (1991) and a validation study conducted by Balls et al. (1995). Three laboratories participated in the CEC (1991) collaborative study and four laboratories participated in the Balls et al. (1995) validation effort. In the CEC (1991) study, each substance tested was assigned a EU classification (R41, R36, or Not Labeled) based on *in vivo* rabbit eye test results. However, due to the lack of individual Draize rabbit eye test scores, a reliability assessment for the CEC (1991) study using the EPA (EPA 2003) or GHS (UN 2007) classification systems was not possible. The Balls et al. (1995) data were used for an evaluation of the interlaboratory reproducibility of the IRE test method according to the EPA, EU, and GHS classification systems (EPA 2003; EU 2001; UN 2007).

6.2.5 Animal Welfare Considerations

The IRE test method reduces animal use by obtaining eyes from rabbits raised for food or rabbits sacrificed after use in other laboratory procedures that do not adversely affect the eye. The IRE test method is a refinement of the *in vivo* rabbit eye test in that the animals are sacrificed prior to application of the test substance and, therefore, the animals do not experience pain and suffering when an ocular irritant is directly applied to the eye. Furthermore, because the IRE test method was adapted from the Draize rabbit eye test specifically to reduce the need for live animals for ocular irritation testing, pain and suffering of the animals is eliminated and the overall number of animals needed for ocular toxicity screening is reduced.

Although rabbits are required as a source of corneas for the IRE test method, only rabbits sacrificed for food or used for other laboratory purposes are used as eye donors (i.e., no live animals are specifically sacrificed for use in this test method).

7.0 ICCVAM Consideration of Public, SACATM, and ICATM Comments

The ICCVAM evaluation process incorporates a high level of transparency. This process is designed to provide numerous opportunities for stakeholder involvement, including submitting written public comments and providing oral comments at ICCVAM independent peer review panel meetings and SACATM meetings. **Table 7-1** lists the nine opportunities for public comments that were provided during the ICCVAM evaluation of the validation status of alternative ocular safety testing methods and approaches. The number of public comments received in response to each of the opportunities is also indicated. Thirty-seven comments were submitted. Comments received in response to or related to *Federal Register* notices (**Appendix H**) are also available on the NICEATM-ICCVAM website.[16] The following sections, delineated by *Federal Register* notice, briefly discuss the public comments received.

Table 7-1 Opportunities for Public Comments

Opportunities for Public Comments	Date	Number of Public Comments Received
70 FR 13512: Request for Data on Non-Animal Methods and Approaches for Determining Skin and Eye Irritation Potential of Antimicrobial Cleaning Product Formulations; Request for Nominations for an Independent Expert Panel	March 21, 2005	0
72 FR 26396: Request for Data on the Use of Topical Anesthetics and Systemic Analgesics for *In Vivo* Eye Irritation Testing	May 9, 2007	1
72 FR 31582: Request for Ocular Irritancy Test Data From Human, Rabbit, and *In Vitro* Studies Using Standardized Testing Methods	June 7, 2007	0
73 FR 18535: Non-Animal Methods and Approach for Evaluating Eye Irritation Potential for Antimicrobial Cleaning Products (AMCPs): Request for Nominations for an Independent Expert Panel and Submission of Relevant Data	April 4, 2008	12
74 FR 14556: Announcement of an Independent Scientific Peer Review Panel on Alternative Ocular Safety Testing Methods; Availability of Draft Background Review Documents (BRD); Request for Comments	March 31, 2009	8
74 FR 19562: Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM)	April 29, 2009	2
Independent Scientific Peer Review Panel Meeting: Alternative Ocular Safety Testing Methods	May 19–21, 2009	12
SACATM Meeting, Arlington Hilton, Arlington, VA	June 25–26, 2009	2
74 FR 33444: Independent Scientific Peer Review Panel Report: Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches; Notice of Availability and Request for Public Comments	July 13, 2009	0

[16] Available at http://ntp-apps.niehs.nih.gov/iccvambp/searchPubCom.cfm

7.1 Public Comments in Response to 70 FR 13512 (March 21, 2005) Request for Data on Non-Animal Methods and Approaches for Determining Skin and Eye Irritation Potential of Antimicrobial Cleaning Product Formulations; Request for Nominations for an Independent Expert Panel

NICEATM requested (1) submission of data that would assist in evaluating the validation status of non-animal methods and approaches used for determining the skin and eye irritation potential of AMCP formulations to meet regulatory hazard classification and labeling purposes and (2) nominations of expert scientists to serve as members of an independent peer review panel.

No data or nominations were received in response to this *Federal Register* notice.

7.2 Public Comments in Response to 72 FR 26396 (May 9, 2007) Request for Data on the Use of Topical Anesthetics and Systemic Analgesics for *In Vivo* Eye Irritation Testing

NICEATM requested submission of (1) data and information on the use of topical anesthetics and systemic analgesics for alleviating pain and distress in rabbits during eye irritation testing and (2) information about other procedures and strategies that may reduce or eliminate pain and distress associated with *in vivo* eye irritation methods.

NICEATM received 1 comment in response to this *Federal Register* notice. This comment was not relevant to *in vitro* ocular safety test methods.

7.3 Public Comments in Response to 72 FR 31582 (June 7, 2007) Request for Ocular Irritancy Test Data From Human, Rabbit, and *In Vitro* Studies Using Standardized Testing Methods

NICEATM requested data on substances tested for ocular irritancy in humans, rabbits, and/or *in vitro* to be used to:

- Review the state of the science in regard to the availability of accurate and reliable *in vitro* test methods for assessing the range of potential ocular irritation activity, including whether ocular damage is reversible or not
- Expand NICEATM's high-quality ocular toxicity database. *In vitro* test methods for which data are sought include but are not limited to (1) the bovine corneal opacity and permeability test, (2) the isolated rabbit eye test, (3) the isolated chicken eye test, and (4) the hen's egg test–chorioallantoic membrane.

No data or information were received in response to this *Federal Register* notice.

7.4 Public Comments in Response to 73 FR 18535 (April 4, 2008) Non-Animal Methods and Approach for Evaluating Eye Irritation Potential for Antimicrobial Cleaning Products (AMCPs): Request for Nominations for an Independent Expert Panel and Submission of Relevant Data

NICEATM requested the following:

- Nominations of expert scientists to serve as members of an independent peer review panel
- Submission of relevant data and information on AMCPs or related substances obtained from (1) human testing or experience, including reports from accidental exposures, and (2) rabbit testing using the standard eye test or the LVET
- *In vitro* ocular safety test methods such as the bovine corneal opacity and permeability test method, the Cytosensor Microphysiometer test method, and the EpiOcular test method, including data supporting the accuracy and reproducibility of these methods

In response to this *Federal Register* notice, NICEATM received 12 comments, including nominations of 20 potential panelists. The nominees were included in the database of experts from which the Panel was selected. No additional data were received.

7.5 Public Comments in Response to 74 FR 14556 (March 31, 2009) Announcement of an Independent Scientific Peer Review Panel on Alternative Ocular Safety Testing Methods; Availability of Draft Background Review Documents (BRD); Request for Comments

NICEATM requested public comments on the draft BRDs, SRDs, and draft ICCVAM test method recommendations that were provided to an independent scientific peer review panel meeting (May 19–21, 2009). These documents summarized the current validation status of several test methods and testing strategies for identifying potential ocular irritants. The test methods and testing strategies included the following:

- A testing strategy that proposes the use of three *in vitro* test methods to assess the eye irritation potential of AMCPs
- Four *in vitro* test methods for identifying moderate (EPA Category II, UN Globally Harmonized System of Classification and Labelling of Chemicals [GHS] Category 2A) and mild (EPA Category III, GHS Category 2B) ocular irritants and substances not classified as ocular irritants (EPA Category IV, GHS Not Classified)
- The *in vivo* LVET
- A proposal for the routine use of topical anesthetics, systemic analgesics, and earlier humane endpoints to avoid and minimize pain and distress during *in vivo* ocular irritation testing

NICEATM received 20 comments in response to this *Federal Register* notice. Eight written comments were received before the Panel meeting, and 12 oral comments were provided at the Panel meeting. Of these comments, 10 were relevant to *in vitro* ocular safety test methods.

Public Responses (written)

HET-CAM—Two written comments were relevant to the HET-CAM test method.

Comment:
One commenter emphasized the importance of establishing one specific protocol and specific endpoints to be used for the HET-CAM test method. Based on a database of 145 substances tested in both the HET-CAM and Draize test methods, the commenter reported that the HET-CAM test method was not useful to identify water-soluble substances as severe irritants in a tiered-testing strategy. However, the HET-CAM might be applicable for excluding severe ocular irritants among water-insoluble substances.

ICCVAM Response:
ICCVAM recommends that additional studies should be conducted to further optimize the HET-CAM prediction models and the decision criteria that would be used to identify ocular corrosives and severe irritants (EPA Category I, EU R41, GHS Category 1). Such studies could potentially improve the usefulness of the HET-CAM test method for identifying these types of substances.

Comment:
Another commenter made the following comments on the HET-CAM draft BRD: (1) the terminology describing the endpoints used in the HET-CAM test method needs to be clarified, (2) the HET-CAM validation database appears to have some inconsistencies with regard to chemical class, (3) the HET-CAM validation database needs to be evaluated by solubility, and (4) the method for counting the days of embryonic development needs to be clarified.

ICCVAM Response:
The text was clarified and supporting references were provided in the final BRD (**Appendix E**) for the endpoints used in the HET-CAM test method. With regard to chemical class, the classifications in the BRD are based on the National Library of Medicine MeSH chemical classification system. Additional details for counting the days of embryonic development were provided in the final BRD.

ICE— One written comment was relevant to the ICE test method.

Comment:
The main point provided by the commenter is that the selection criteria set forth by ICCVAM for classification purposes of substances tested with the ICE test method are inappropriate. The commenter states that additional data from studies that were terminated earlier than 21 days after treatment or compounds lacking an *in vivo* eye irritation study because of proven *in vivo* skin corrosivity should be considered. The commenter also addressed the variability of the Draize rabbit eye test and expressed concern that the Draize rabbit eye test (i.e., OECD TG 405) has no standardized exposure regimen.

ICCVAM Response:
The performance of the ICE test method was reevaluated after including skin corrosivity test results (n = 8) and corrections to the classification of specific test substances, where appropriate based on the additional data and information provided by the commenter. These changes are reflected in the ICE final BRD (**Appendix F**). However, the addition and modification of these test results did not significantly impact the performance of the ICE test method or the conclusions and recommendations of the Panel or ICCVAM.

HCE— One written comment was specific to the human corneal epithelial cell (HCE) model.

Comment:
The commenter provided comments on an ATLA journal article (Eskes et al. 2005) that described the human corneal epithelial cell (HCE) model and a list of key references omitted from that review.

ICCVAM Response:
Although the HCE model was not part of the current evaluation, ICCVAM welcomes comments on alternative *in vitro* test methods at any time. ICCVAM encourages the submission of data for the HCE model for future evaluation of its validation status.

Public Responses, Oral
Twelve oral public comments were provided at the Panel meeting (May 19-21, 2009).

HET-CAM— One commenter remarked specifically on the HET-CAM test method.

Comment:
One commenter indicated that the false negatives using the EPA classification system, which are Not Classified using the GHS classification system, result because the EPA classification system categorizes substances based upon the most severe category observed among the test rabbits (i.e., hazard classification is not based on the majority classification among rabbits tested). The commenter noted that because the types of formulations regulated by EPA are not present in the database, the EPA classification system should not be given too much weight.

ICCVAM Response:
Until the GHS classification system is formally adopted, ICCVAM will continue to consider all relevant hazard classification systems (i.e., EPA, EU, and GHS) when evaluating the usefulness and limitations of an *in vitro* test method.

ICE— One commenter remarked specifically on the ICE test method.

Comment:
One commenter indicated that the variability of the ICE test method was similar to that of the Draize rabbit eye test. The commenter stated that the ICE test method should not be held to a higher standard than the Draize rabbit eye test and noted that the concordance among laboratories was reasonable.

ICCVAM Response:
All *in vitro* test methods are evaluated according to the *ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods* (ICCVAM 2003), including a comparison to the currently accepted regulatory test method as a reference.

BCOP— Three commenters remarked specifically on the BCOP test method.

Comment:
One commenter indicated that the performance of the BCOP test method was unlikely to improve based on the lack of reproducibility with the Draize rabbit eye test in the mild and moderate categories. The commenter stated that results from Weil and Scala (1971) show that the extremes (i.e., corrosives/severe irritants and substances not labeled as irritants) are reproducible, but the mild and moderate levels of ocular irritation are highly variable. The commenter referenced the AMCP BRD that includes an analysis of the impact on the ocular hazard category when the results of a six-rabbit Draize test are randomly sampled for a three-rabbit test.

ICCVAM Response:
The Draize rabbit eye test (Draize et al. 1944) has a long history of demonstrated protection of public health and therefore, U.S. and international regulatory agencies currently use this test to identify potential ocular hazards. Alternatives are accepted only when they demonstrate the ability to provide equal or better protection than the reference test method. Given the uncertainty of the results associated with the BCOP test method for substances in the mild/moderate irritancy range, the BCOP test method cannot be considered a complete replacement at this time.

Comment:
A second commenter stated that damaged eyes are quickly removed and excluded from the BCOP test method and that Gautheron et al. (1992) used both fresh eyes and eyes maintained at 4ºC and found no differences in results. The commenter also asked the Panel to reconsider the use of a histopathology evaluation in the BCOP test method.

ICCVAM Response:
ICCVAM previously evaluated and recommended the BCOP test method for the identification of corrosive/severe ocular irritants (ICCVAM 2006e). In the current evaluation, ICCVAM, along with the Panel, recommends the BCOP test method for the identification of substances not labeled as irritants. Furthermore, the final ICCVAM recommendations state that a histopathological evaluation of the corneal tissue, using standardized procedures, should be included when the BCOP test method is conducted. Such data will allow for development of decision criteria and future assessments on the usefulness of this endpoint for classifying and labeling substances, especially those that may otherwise produce borderline or false negative results.

Comment:
A third commenter discussed the “top-down” (i.e., screening for corrosives/severe irritants) and “bottom-up” (i.e., screening for substances not labeled as irritants) approaches using the ICE and

BCOP test methods. The commenter stated that ECVAM is developing a paper to recommend the use of these proposed testing strategies for both ICE and BCOP, where substances could be tested in the BCOP or ICE test methods in order to identify corrosives/severe irritants or substances not labeled as irritants without using an animal test.

ICCVAM Response:
ICCVAM currently recommends the ICE and BCOP test methods for use in a tiered-testing strategy, where positive substances can be classified as ocular corrosives and severe irritants without the need for animal testing (ICCVAM 2006e). However, identification of nonsevere ocular irritants and substances not labeled as irritants would require another test that has been demonstrated as scientifically validated for identifying such substances.

Comment:
One commenter questioned the need for performance standards for the CM test method, given that the Panel did not recommend performance standards for the BCOP and ICE test methods.

ICCVAM Response:
The final ICCAM recommendations state that the development of performance standards for the CM test method is not warranted at this time.

7.6 Public Comments in Response to 74 FR 19562 (April 29, 2009) Meeting of the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM)

NICEATM announced the SACATM meeting (June 25–26, 2009) and requested written and public oral comments on the agenda topics.

NICEATM received four comments. Two written comments were received before the meeting, and two oral comments were provided at the SACATM meeting. None of these comments were relevant to *in vitro* ocular safety test methods.

SACATM Response:
In general, SACATM was pleased with the Panel report. One SACATM member expressed the need for harmonization in the assessment of performance standards. Another SACATM member said the focus should be on the GHS system because it will ultimately be adopted. Another SACATM member expressed concern regarding the availability of the Cytosensor Microphysiometer.

7.7 Public Comments in Response to 74 FR 33444 (July 13, 2009) Independent Scientific Peer Review Panel Report: Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches; Notice of Availability and Request for Public Comments

NICEATM requested submission of written public comments on the independent scientific peer review panel report.

No public comments were received.

7.8 Comments Received from ICATM Validation Organizations

In accordance with the International Cooperation on Alternative Test Methods (ICATM), each participating organization (i.e., ECVAM, Health Canada, and JaCVAM) was given the opportunity to comment prior to finalizing these recommendations. All ICATM partners agreed with these recommendations with one exception noted from ECVAM regarding the usefulness and limitations of the BCOP test method (see **Appendix H4**). ECVAM agreed with ICCVAM that the BCOP test method should not be recommended for the identification of substances not labeled as irritants under the EPA and FHSA classification system. However, they did not agree with ICCVAM's concerns

about the underprediction that would occur with the use of the BCOP test method under the GHS classification system compared to the EPA and FHSA systems currently used in the United States. Rather, ECVAM expressed the view that the predictivity of a test method should be calculated independently for each classification, without regard to the nature, severity, and duration of eye injuries that were the basis for ICCVAM's concerns, and that serve as the basis for classification as eye hazards using current U.S. classification criteria. Thus, ECVAM considered the calculated predictivity of the BCOP test method for available test data (0% [0/97] false negatives) to support its use for identifying substances not labeled as irritants when compared strictly to the eye hazard criteria in the current GHS classification system. While ECVAM also noted that their recommendations would be in line with the ICCVAM Peer Review Panel, as stated in **Section 2.1.1**, the Peer Panel deliberations preceded the NICEATM evaluation of the GHS classification system that indicates an estimated 30% or more of substances requiring labeling for eye irritation hazard according to current U.S. hazard classification requirements will not be labeled as eye irritation hazards by the current GHS criteria. ECVAM disagrees with the ICCVAM concern that, "*the nature, severity, and duration of these eye injuries suggest the potential to cause human injury,"* because they state that there is no empirical evidence to substantiate that there should in fact be such a concern. While ICCVAM was not able to find any human accidental exposure data for the chemicals that will no longer be labeled as eye hazards, there also are no human exposure or test data to suggest that these chemicals, which produced significant eye injuries in rabbits, will not produce significant injuries to human eyes.

NICEATM has searched numerous databases (i.e., OSHA, CPSC, EPA) for human eye injury data for these chemicals, but no relevant data have been identified. U.S. Federal law requires agencies to determine that new test methods recommended by ICCVAM generate data that are at least equivalent to data generated by current test methods required or recommended by each agency for hazard identification purposes. Therefore, until the issues associated with the GHS system as outlined above are further discussed, ICCVAM is deferring final recommendations on the usefulness and limitations of using BCOP as a screening test to identify substances not labeled as irritants according to the GHS classification system. At such time that relevant human testing or exposure data becomes available, ICCVAM will revisit this issue. In the meantime, the Scientific Advisory Committee on Alternative Toxicological Methods will be asked to comment at their June 2010 meeting about the extent that reversible chemically induced eye injuries in rabbits might indicate the potential for injury to humans. Minutes of this meeting will be available on the NTP website at: http://ntp.niehs.nih.gov/index.cfm?objectid=720165EC-BDB7-CEBA-F517D1DEE4D7D129.

8.0 References

Balls M, Botham PA, Bruner LH, Spielmann H. 1995. The EC/HO international validation study on alternatives to the Draize eye irritation test. Toxicol In Vitro 9:871–929.

Brantom PG, Bruner LH, Chamberlain M, DeSilva O, Dupuis J, Earl LK, et al. 1997. A summary report of the COLIPA International Validation Study on Alternatives to the Draize Rabbit Eye Irritation Test. Toxicol In Vitro 11:141–179.

Burton AB, York M, Lawrence RS. 1981. The *in vitro* assessment of severe eye irritants. Food Cosmet Toxicol 19:471–480.

CEC. 1991. Collaborative Study on the Evaluation of Alternative Methods to the Eye Irritation Test. Doc. XI/632/91/V/E/1/131/91 Part I and II. Brussels:Commission of the European Communities.

Cooper KJ, Earl LK, Harbell J, Raabe H. 2001. Prediction of ocular irritancy of prototype shampoo formulations by the isolated rabbit eye (IRE) test and bovine corneal opacity and permeability (BCOP) assay. Toxicol In Vitro 15:95–103.

CPSC. 2003. Commercial practices. Hazardous Substances and Articles; Administration and Enforcement Regulations. 16 CFR 1500.42.

Draize J, Woodard G, Calvery H. 1944. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharmacol Exp Ther 82:377–390.

ECETOC. 1998. Eye Irritation – Reference Chemicals Data Bank. Technical Report No. 48(2). Brussels:European Centre for Ecotoxicology and Toxicology of Chemicals.

EPA. 2003. Label Review Manual. 3rd ed. EPA737-B-96-001. Washington, DC:U.S. Environmental Protection Agency.

ESAC. 2009. Statement on the scientific validity of cytotoxicity/cell-function based in vitro assays for eye irritation testing. Ispra, Italy:European Commission. Available: http://ecvam.jrc.it/index.htm.

Eskes et al. 2005. Eye irritation. Altern Lab Anim 33(Suppl. 1):47-81.

EU. 2001. Commission Directive 2001/59/EC of 6 August 2001 adapting to technical progress for the 28th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. Official Journal of the European Communities L255:1–333.

Federal Hazardous Substances Act (FHSA). 2005. Public Law 86-613.

Gautheron P, Dukic M, Alix D, Sina JF. 1992. Bovine corneal opacity and permeability test: An in vitro assay of ocular irritancy. Fundam Appl Toxicol 18:442-449.

Gautheron P, Giroux J, Cottin M, Audegond L, Morilla A, Mayordomo-Blanco L, et al. 1994. Interlaboratory assessment of the bovine corneal opacity and permeability (BCOP) assay. Toxicol In Vitro 8:381–392.

Gettings SD, Lordo RA, Demetrulias J, Feder PI, Hintze KL. 1996. Comparison of low-volume, Draize and in vitro eye irritation test data. I. Hydroalcoholic formulations. Food Chem Toxicol 34:737–749.

Guerriero FJ, Seaman CW, Olson MJ, Guest R, Whittingham A. 2004. Retrospective assessment of the rabbit enucleated eye test (REET) as a screen to refine worker safety studies [Abstract No. 1282]. Toxicol Sci (supplement) 78(1-S).

Hagino S, Kinoshita S, Tani N, Nakamura T, Ono N, Konishi K, Iimura H, Kojima H, Ohno Y. 1999. Interlaboratory validation of *in vitro* eye irritation tests for cosmetic ingredients. (2) Chorioallantoic membrane (CAM) test. Toxicol In Vitro 13:99-113.

ICCVAM. 2003. ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods. NIH Publication No. 03-4508. Research Triangle Park, NC:National Institute of Environmental Health Sciences.

ICCVAM. 2006a. Background Review Document: Current Status of *In Vitro* Test Methods for Identifying Ocular Corrosives and Severe Irritants: Bovine Corneal Opacity and Permeability Test Method. NIH Publication No. 06-4512. Research Triangle Park, NC:National Institute of Environmental Health Sciences.

ICCVAM. 2006b. Background Review Document: Current Status of *In Vitro* Test Methods for Identifying Ocular Corrosives and Severe Irritants: Hen's Egg Test–Chorioallantoic Membrane Test Method. NIH Publication No. 06-4515. Research Triangle Park, NC:National Institute of Environmental Health Sciences.

ICCVAM. 2006c. Background Review Document: Current Status of *In Vitro* Test Methods for Identifying Ocular Corrosives and Severe Irritants: Isolated Chicken Eye Test Method. NIH Publication No. 06-4513. Research Triangle Park, NC:National Institute of Environmental Health Sciences.

ICCVAM. 2006d. Background Review Document: Current Status of *In Vitro* Test Methods for Identifying Ocular Corrosives and Severe Irritants: Isolated Rabbit Eye Test Method. NIH Publication No. 06-4514. Research Triangle Park, NC:National Institute of Environmental Health Sciences.

ICCVAM. 2006e. ICCVAM Test Method Evaluation Report: *In Vitro* Ocular Toxicity Test Methods for Identifying Severe Irritants and Corrosives. NIH Publication No. 07-4517. Research Triangle Park, NC:National Institute of Environmental Health Sciences.

Jones PA, Budynsky E, Cooper KJ, Decker D, Griffiths HA, Fentem JH. 2001. Comparative evaluation of five *in vitro* tests for assessing the eye irritation potential of hair-care products. ATLA 29:669–692.

Koëter BWM, Prinsen MK. 1985. Comparison of *in vivo* and *in vitro* eye irritancy test systems: a study with 34 substances. In: Alternative Methods in Toxicology (Goldberg AM, ed). Vol 3. New York:Mary Ann Liebert, 571–579.

Luepke N. 1985. Hen's egg chorioallantoic membrane test for irritation potential. Food Chem Toxic 23:287-291.

Maurer JK, Parker RD, Jester JV. 2002. Extent of corneal injury as the mechanistic basis for ocular irritation: key findings and recommendations for the development of alternative assays. Reg Tox Pharmacol 36:106-117.

OECD. 1999. Detailed Review Document Number 14. Classification systems for eye irritation/corrosion in OECD member countries. ENV/JM/MONO(99)4. In: OECD Series on Testing and Assessment. Available: http://www.olis.oecd.org/olis/1999doc.nsf/LinkTo/env-jm-mono%2899%294.

OECD. 2009a. Test Guideline 437. Bovine corneal opacity and permeability test method for identifying ocular corrosives and severe irritants, adopted September 7, 2009. In: OECD Guidelines for Testing of Chemicals. Paris:Organisation for Economic Co-operation and Development.

OECD. 2009b. Test Guideline 438. Isolated chicken eye test method for identifying ocular corrosives and severe irritants, adopted September 7, 2009. In: OECD Guidelines for Testing of Chemicals. Paris:Organisation for Economic Co-operation and Development.

Southee JA. 1998. Evaluation of the Prevalidation Process. Part 2, Final Report. Vol. 2. The Bovine Corneal Opacity and Permeability (BCOP) Assay. European Community Contract No. 11279-95-10F 1ED ISP GB.

UN. 2007. Globally Harmonised System of Classification and Labelling of Chemicals (GHS). New York:United Nations Publications.

Weil CS, Scala RA. 1971. Study of intra- and interlaboratory variability in the results of rabbit eye and skin irritation tests. Toxicol Appl Pharmacol.19:276-360.

This page intentionally left blank

Appendix A

ICCVAM Evaluation Timeline

This page intentionally left blank

ICCVAM Evaluation Timeline

June 7, 2007	*Federal Register* Notice (72 FR 31582) – Request for Ocular Irritancy Test Data From Human, Rabbit, and In Vitro Studies Using Standardized Test Methods.
November 26, 2007	*Federal Register* Notice (72 FR 65964) – Availability of Test Method Evaluation Report and Final Background Review Documents on In Vitro Ocular Toxicity Test Methods for Identifying Severe Irritants and Corrosives; ICCVAM Test Method Recommendations to Federal Agencies.
April 4, 2008	*Federal Register* Notice (73 FR 18535) – Non-Animal Methods and Approach for Evaluating Eye Irritation Potential for Antimicrobial Cleaning Products (AMCPs): Request for Nominations for an Independent Expert Panel and Submission of Relevant Data. Included request for information on AMCPs or related substances from: 1. Human testing or accidental exposures 2. The standard or low volume eye test 3. *In vitro* test methods (i.e., bovine corneal opacity and permeability, Cytosensor® Microphysiometer, EpiOcular test).
March 31, 2009	*Federal Register* Notice (74 FR 14556) – Announcement of an Independent Scientific Peer Review Panel Meeting on the Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches; Availability of Draft Background Review Documents (BRD) and Summary Review Documents (SRD); Request for Comments.
May 19-21, 2009	Independent Scientific Peer Review Panel holds a public meeting, with opportunity for public comments, at CPSC Headquarters in Bethesda, MD. The Panel was charged with reviewing the current validation status of alternative ocular safety testing methods and strategies, and commenting on the extent to which the information in the draft BRD and SRD supported the draft ICCVAM test method recommendations.

June 25-26, 2009	SACATM public meeting, SACATM and public comments on the draft Panel conclusions and recommendations.
July 13, 2009	*Federal Register* notice (74 FR 33444) – Independent Scientific Peer Review Panel Report: Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches: Notice of Availability and Request for Public Comments.
October 29, 2009	ICCVAM endorses the Test Method Evaluation Report, which includes the final Background Review Documents.

Appendix B

ICCVAM-Recommended Test Method Protocols

B1 ICCVAM-Recommended Protocol for Future Studies Using the Bovine Corneal Opacity And Permeability (BCOP) Test Method B-3

B2 ICCVAM-Recommended Protocol for Future Studies Using the Cytosensor Microphysiometer (CM) Test Method B-19

B3 ICCVAM-Recommended Protocol for Future Studies Using the Hen's Egg Test–Chorioallantoic Membrane (HET-CAM) Test Method B-29

B4 ICCVAM-Recommended Protocol for Future Studies Using the Isolated Chicken Eye (ICE) Test Method B-39

B5 ICCVAM-Recommended Protocol for Future Studies Using the Isolated Rabbit Eye (IRE) Test Method B-51

This page intentionally left blank

Appendix B1

ICCVAM-Recommended Protocol for Future Studies Using the Bovine Corneal Opacity and Permeability (BCOP) Test Method

This page intentionally left blank

Preface

This proposed protocol for measuring corneal damage was developed following a comprehensive test method evaluation process conducted by ICCVAM, which included an international independent scientific peer review of the validation status and scientific validity of the BCOP (ICCVAM 2006a,b). It is based primarily on information obtained from (1) the Institute for In Vitro Sciences, Inc. (IIVS), a nonprofit foundation that has performed the BCOP assay since 1997 in a Good Laboratory Practice (GLP)-compliant testing facility and (2) INVITTOX Protocol 124 (1999), which represents the protocol used for the European Community sponsored prevalidation study of the BCOP assay conducted in 1997–1998. Both of these protocols are based on the BCOP assay methodology first reported by Gautheron et al. (1992). Future studies using the BCOP test method could include further characterization of the usefulness or limitations of the BCOP in a weight-of-evidence approach for regulatory decision-making. Users should be aware that the proposed test method protocol could be revised based on any additional optimization and/or validation studies that are conducted in the future. ICCVAM recommends that test method users consult the NICEATM–ICCVAM website (http://iccvam.niehs.nih.gov/) to ensure use of the most current test method protocol.

1.0 Purpose and Applicability

The purpose of this protocol is to describe the procedures used to evaluate the potential ocular corrosivity or severe irritancy of a test substance as measured by its ability to induce opacity and increase permeability in an isolated bovine cornea. Effects are measured by (1) decreased light transmission through the cornea (opacity); (2) increased passage of sodium fluorescein dye through the cornea (permeability); and (3) evaluation of fixed and sectioned tissue at the light microscopic level, if applicable. The opacity and permeability assessments of the cornea following exposure to a test substance are considered individually and also combined to derive an *in vitro* irritancy score, which is used to classify the irritancy level of the test substance. Histological evaluation of the corneas can be useful for identifying damage in tissue layers that does not produce significant opacity or permeability.

The focus of this protocol is on the use of the BCOP test method for the detection of ocular corrosives and severe irritants, as defined by the U.S. Environmental Protection Agency (EPA; EPA 2003a), European Union (EU; EU 2001), and United Nations Globally Harmonized System (GHS) of Classification and Labelling of Chemicals (UN 2007). Substances other than ocular corrosives and severe irritants (e.g., substances not labeled as irritants and mild/moderate ocular irritants) have been tested using this protocol; however, the BCOP test method is not currently considered to be adequately validated for these classes of ocular irritancy as defined by EPA (2003a), EU (2001), and GHS (UN 2007).

2.0 Safety and Operating Precautions

All procedures with bovine eyes and bovine corneas should follow the institution's applicable regulations and procedures for handling animal substances, which include, but are not limited to, tissues and tissue fluids. Universal laboratory precautions are recommended, including the use of laboratory coats, eye protection, and gloves. If available, additional precautions required for specific study substances should be identified in the Material Safety Data Sheet for that substance.

3.0 Materials, Equipment, and Supplies

3.1 Source of Bovine Eyes

Eyes from cattle are obtained from an abattoir located within close proximity of the testing facility. The cattle type (breed not specified) can be cows, heifers, steers, or bulls. Because cattle have a wide range of weights depending on breed, age, and sex, there is no recommended weight for the animal at the time of sacrifice.

Eyes from very old cattle are not recommended because the corneas tend to have a greater horizontal corneal diameter and vertical corneal thickness that could affect assay performance (Doughty et al. 1995; Harbell J, personal communication). Additionally, eyes from calves are not recommended since their corneal thickness and corneal diameter are considerably less than that of eyes from adult cattle.

3.2 Equipment and Supplies

- Corneal holders[1]
- Dissection equipment (scissors, scalpels, forceps)
- Electric screwdriver
- Falcon tubes (50 mL)
- Incubator or water bath
- Liquinox (or equivalent)
- Microplate reader or UV/VIS spectrophotometer
- Micropipettors and pipette tips
- Opacitometer
- Petri dishes
- Plastic containers for collection and transport of eyes
- Sample tubes (5 mL, glass) for permeability determination
- Spatula
- Specialized window-locking ring screwdriver
- Standard tissue culture and laboratory equipment
- Sterile deionized water
- Syringes (10 mL) and blunt tip needles (19 Gauge)
- Vacuum pump
- Volumetric flasks
- 96 well plates (polystyrene) or cuvettes of an appropriate size for UV/VIS spectrophotometer

3.3 Chemicals

- Ethanol (200 proof, absolute, anhydrous, ACS/USP grade)
- Imidazole
- Penicillin
- Sodium chloride
- Sodium fluorescein
- Streptomycin

[1] Users should be aware of a proposed corneal holder developed by Ubels et al. (2002). The ICCVAM Test Method Evaluation Report (2006b) recommends, "Studies should be conducted to evaluate the impact of using a corneal holder that maintains normal curvature (e.g., the corneal mounting system designed by Ubels et al. 2002) on accuracy and/or reliability of the BCOP test method."

3.4 Solutions

Follow the manufacturer's recommendations with regard to storage temperature and shelf life of stock solutions. Prepare assay solutions volumetrically.

- 0.9% (w/v) NaCl in sterile deionized water (saline).
- 1X Hanks' Balanced Salt Solution with Ca^{++} and Mg^{++} (HBSS) containing 100 IU/mL penicillin and 100 µg/mL streptomycin.
- Dulbecco's Phosphate Buffered Saline (DPBS).
- Eagle's Minimum Essential Medium without phenol red containing 1% (v/v) Fetal Bovine Serum (complete MEM), warmed to 32˚C.
- Eagle's Minimum Essential Medium with phenol red containing 1% Fetal Bovine Serum (complete MEM with phenol red, used only for rinsing test substances), warmed to 32˚C.
- Sodium fluorescein (Na-fluorescein) diluted in DPBS to 4 mg/mL for liquid test articles or 5 mg/mL for solid test articles.

4.0 Test Substance Preparation

All test substance solutions should be prepared fresh on the day of use.

4.1 Nonsurfactant Liquid Test Substances

Liquid test substances are usually tested undiluted. However, if prescribed, dilutions of aqueous soluble test substances should be prepared in 0.9% sodium chloride.

4.2 Nonsurfactant Solid Test Substances

Nonsurfactant solid test substances should be prepared as 20% (w/v) solutions or suspensions in 0.9% sodium chloride.

4.3 Surfactants

Solid and concentrated liquid surfactants should be prepared and tested as a 10% (w/v, v/v) dilution or suspension in 0.9% sodium chloride.

4.4 Surfactant Preparations

Surfactant-based preparations (e.g., product formulations) are usually tested neat, or can be diluted in 0.9% sodium chloride, with justification of the selected dilution.

5.0 Controls

5.1 Negative Control

When testing a liquid substance at 100%, a concurrent negative control (e.g., 0.9% sodium chloride) is included to detect nonspecific changes in the test system, as well as to provide a baseline for the assay endpoints.

5.2 Solvent/Vehicle Control

When testing a diluted liquid, surfactant, or solid, a concurrent solvent/vehicle control is included to detect nonspecific changes in the test system, as well as to provide a baseline for the assay endpoints.

5.3 Positive Control

A known ocular irritant is included as a concurrent positive control in each experiment to verify that an appropriate response is induced. As the BCOP assay is being used to identify corrosive or severe irritants, ideally the positive control should be a reference substance that induces a severe response in this test method. However, to ensure that variability in the positive control response across time can be assessed, the magnitude of irritant response should not be excessive.

Examples of positive controls for liquid test substances are 1% sodium hydroxide or 10% dimethylformamide. An example of a positive control for solid test substances is 20% (weight to volume) imidazole in 0.9% sodium chloride solution.

5.4 Benchmark Substances (if appropriate)

Benchmark substances are useful for evaluating the ocular irritancy potential of unknown chemicals of a specific chemical or product class, or for evaluating the relative irritancy potential of an ocular irritant within a specific range of irritant responses. Appropriate benchmark substances should have the following properties:

- A consistent and reliable source(s)
- Structural and functional similarity to the class of the substance being tested
- Known physical/chemical characteristics
- Supporting data on known effects in the *in vivo* rabbit eye test
- Known potency in the range of the desired response

6.0 Experimental Design

6.1 Collection and Transport Conditions of Bovine Eyes

Bovine eyes are typically obtained from a local cattle abattoir, where the eyes are excised as soon as possible after sacrifice. Care should be taken to avoid damaging the cornea during the enucleation procedure. Eyes are collected in a suitable container in which they are immersed in HBSS containing the antibiotics penicillin (100 IU/mL) and streptomycin (100 µg/mL) The container is maintained on ice at all times throughout collection of the eyes and transportation to the testing facility (NOTE: antibiotics may not be necessary if the eyes are kept below 4°C throughout transport). The eyes are used within five hours of sacrifice.

Under conditions where contamination of the bovine eyes with yeast occurs, immersion of the eyes in HBSS containing fungizone should be evaluated.

6.2 Preparation of Corneas

a. Carefully examine all eyes macroscopically. Those exhibiting unacceptable defects, such as opacity, scratches, pigmentation, and neovascularization are rejected.

b. Carefully remove the cornea from each selected eye by making an incision with a scalpel 2 to 3 mm outside the cornea, then by cutting around the cornea with dissection scissors, leaving a rim of sclera to facilitate handling. Carefully peel off the iris and lens, ensuring no fragments of these tissues are remaining on the cornea. Take care to avoid damaging the corneal epithelium and endothelium during dissection.

c. Store the isolated corneas in a petri dish containing HBSS until they are mounted in holders. Examine the corneas before use, and discard those with defects.

d. Mount the corneas in holders (one cornea per holder) by placing the endothelial side of the cornea against the O-ring of the posterior chamber. Place the anterior chamber over the cornea and join the chambers together by tightening the chamber screws. Care should be taken not to shift the two chambers to avoid damaging the cornea.

e. Fill both chambers with fresh complete MEM (about 5 mL), always filling the posterior chamber first to return the cornea to its natural curvature. Care should be taken when adding or removing liquid from the posterior chamber to avoid the formation of bubbles and to minimize shear forces on the corneal endothelium.

f. Seal each chamber with plugs provided with the holders.

g. Incubate the holders in a vertical position at 32 ± 1°C for at least 60 minutes.

h. At the end of the initial 1-hour incubation period, examine each cornea for defects, such as tears or wrinkling. Discard corneas with any observed defects.

6.3 Control Cornea Selection and Opacity Reading

a. After the 1-hour incubation period, remove the medium from both chambers of each holder (anterior chamber first) and replace with fresh complete MEM.

b. Take and record an initial opacity reading for each cornea, using an opacitometer or equivalent instrument that has been appropriately calibrated according to the manufacturer's specifications. This initial opacity reading will be used to calculate the final opacity value for each cornea. The testing facility should ensure the opacitometer is functioning properly each day it is used.

c. Calculate the average opacity value for all corneas.

d. Select a minimum of three corneas with opacity values close to the average value for all corneas as negative (or solvent/vehicle) control corneas.

e. Corneas that display an initial opacity reading significantly greater (+ 2 standard deviations [SDs]) than the average opacity for all corneas in the batch of eyes collected the day of testing should not be used in the assay.

6.4 Treatment Groups

A minimum of three corneas are treated with each test substance solution or suspension. In addition, three corneas per assay are treated with the positive control and three corneas per assay are treated with the negative control. If a benchmark substance is used the day of testing, three corneas should be treated with the benchmark.

Different treatment methods are used depending on the physical nature and chemical characteristics (liquid or surfactant versus nonsurfactant solid) of the test substance. The controls used depend on which method is used.

6.5 Treatment of Corneas and Opacity Measurements

6.5.1 Closed chamber method for nonviscous to slightly viscous liquid test substances

a. Record the initial opacity readings and label each chamber with the appropriate control or test substance identification. Just prior to treatment, remove the medium from the anterior chamber through the dosing holes using an appropriate aspiration technique (e.g., blunt needle attached to a vacuum pump).

b. Add 0.75 mL of the control or test substance to the anterior chamber through the dosing holes using a micropipettor. The dosing holes are then resealed with the chamber plugs.

c. Rotate the holders such that the corneas are in a horizontal position. The holders should be gently tilted back and forth to ensure a uniform application of the control or test substance over the entire cornea.

d. Incubate the holders in a horizontal position at 32 ± 1°C for 10 ± 1 minutes. If other exposure times are used, justification must be provided.

e. Remove the control or test substance from the anterior chamber through the dosing holes and rinse the epithelium at least three times with approximately 2 to 3 mL of fresh complete MEM with phenol red. Perform one last rinse of the epithelium using fresh complete MEM. If it is not possible to remove all visible signs of the test substance, document the observation in the study notebook. Refill the anterior chamber with fresh complete MEM.

f. Perform a post-treatment opacity reading for each cornea and record the results. Observe each cornea visually and, if applicable, record pertinent observations (e.g., dissimilar opacity patterns, tissue peeling or residual test article).

g. Incubate the holders in a vertical (anterior chamber facing forward) position at 32 ± 1°C for 120 ± 10 minutes. If other post-exposure incubation times are used, justification should be provided.

h. Record a post-incubation opacity reading for each cornea, which will be used to calculate the final corneal opacity value. Observe each cornea visually and record pertinent observations in the study notebook. Special attention is taken to observe dissimilar opacity patterns, tissue peeling or residual test substance, etc.

6.5.2 Open chamber method for semiviscous and viscous liquid test substances and surfactant preparations

a. Record the initial opacity readings and label each chamber with the appropriate control or test article identification. Just prior to treatment, remove the medium from the anterior chamber through the dosing holes.

b. Remove the window-locking ring and glass window from all appropriate anterior chambers and place the holders into a horizontal position (anterior chamber facing up).

c. Add test substance to each chamber successively at a constant rate of 15 to 30 seconds between each chamber. Apply approximately 0.75 mL of the control or test substance (or enough test substance to completely cover the cornea) directly to the epithelial surface of the cornea using a micropipettor or other appropriate device, such as a spatula. Maintain the holders in a horizontal position (anterior chamber up).

d. If necessary, to aid in filling the pipette with substances that are viscous, the test article may first be transferred to a syringe. Insert the pipette tip of the positive displacement pipette into the dispensing tip of the syringe, so that the substance can be loaded into the displacement tip under pressure. Simultaneously, depress the syringe plunger as the pipette piston is drawn upwards. If air bubbles appear in the pipette tip, the test article should be expelled and the process repeated until the tip is filled without air bubbles. This method should be used for any substances that cannot be easily drawn into the pipette (e.g., gels, toothpastes, and face creams).

e. If necessary, immediately upon dosing, slightly tilt the holders to achieve a uniform application of the test article over the entire cornea.

f. After all of the chambers are dosed, replace the glass windows and window-locking rings.

g. Incubate the holders in a horizontal position at 32 ± 1°C for 10 ± 1 minutes. If other exposure incubation times are used, justification should be provided.

h. Prior to the end of the exposure period, remove the window-locking ring and glass window from each appropriate chamber.

i. At the completion of the exposure period, successively rinse each cornea in the exposure group according to the intervals that they were dosed. Using a syringe, add fresh complete MEM with phenol red to the inside wall of the anterior chamber creating a "whirlpool or vortex effect", which causes the test article to be rinsed off the cornea. Take special care not to spray the medium directly onto the cornea. Residual test article that cannot be removed from the cornea by the "whirlpool method" is removed by placing a layer of medium over the cornea (added to the inside wall of the chamber). Spray a gentle stream of medium through the medium layer, directing it towards the residual test article. If after several tries the test article cannot be removed, document this in the study notebook, and proceed to the next step.

j. Once each cornea is completely rinsed of test article, replace the glass window and window-locking ring. Continue rinsing as stated previously for the "closed chamber method" (see Section 6.5.1, step e).

k. Perform a post-treatment opacity reading for each cornea and record the results. Observe each cornea visually and, if applicable, record pertinent observations (e.g., dissimilar opacity patterns, tissue peeling or residual test article).

l. Incubate the holders in a vertical (anterior chamber facing forward) position at 32 ± 1°C for 120 ± 10 minutes. If other post-exposure incubation times are used, justification should be provided.

m. Record a post-incubation opacity reading for each cornea, which will be used to calculate the final corneal opacity value. Observe each cornea visually and record pertinent observations in the study notebook. Special attention is taken to observe dissimilar opacity patterns, tissue peeling or residual test substance, etc.

6.5.3 Solid and liquid surfactant test substances

Surfactant test substances are administered following one of the previously described procedures, with one exception: Surfactant test substances are tested on the cornea as a 10% (w/v) solution or suspension prepared in an appropriate solvent/vehicle (e.g., sterile deionized water).

6.5.4 Solid nonsurfactant test substances

Solid nonsurfactant test substances are administered following one of the previously described procedures, with a few exceptions, which are noted below:

- Solid test substances are tested on the cornea as a 20% (w/v) solution or suspension prepared in an appropriate solvent/vehicle (e.g., sterile deionized water).
- Solid test substances are incubated at 32 ± 1°C for 240 ± 10 minutes.
- There is no post-treatment incubation period. Thus, immediately following the rinsing process, both chambers are refilled (posterior chamber first) with fresh complete MEM,

and the post-treatment opacity readings are taken. During the post-treatment opacity reading, visual observations are performed for each cornea and, if necessary, are recorded in the workbook. Special attention is taken to observe dissimilar opacity patterns, tissue peeling or residual test article, etc. Immediately following these opacity readings and visual observations, the permeability experiment is performed.

6.6 Application of Sodium Fluorescein

Following the final opacity measurement, permeability of the cornea to Na-fluorescein is evaluated. The Na-fluorescein solution is applied to the cornea by one of two methods, depending on the nature of the test substance:

Liquid and surfactant test substances and surfactant preparations:

a. Remove the medium from both chambers (anterior chamber first).

b. Fill the posterior chamber with fresh complete MEM, and add 1 mL of a 4 mg/mL Na-fluorescein solution to the anterior chamber using a micropipettor.

c. Reseal the dosing holes in the top of both chambers with the chamber plugs.

Solid nonsurfactant test substances:

a. Remove the medium from the anterior chamber only and replace with 1 mL of a 5 mg/mL Na-fluorescein solution.

b. Reseal the dosing holes in the top of both chambers with the chamber plugs.

6.7 Permeability Determinations

a. After adding the Na-fluorescein to the anterior chamber and sealing the chambers, rotate the holders into a horizontal position with the anterior chamber facing up. Tilt the holders slightly, if necessary, to achieve a uniform application of the Na-fluorescein over the entire cornea. Incubate the holders in a horizontal position for 90 ± 5 minutes at 32 ± 1°C.

b. After the 90-minute incubation period, remove the medium in the posterior chamber of each holder and place into sample tubes prelabeled according to holder number. It is important to remove most of the medium from the posterior chamber and mix it in the tube so that a representative sample can be obtained for the OD_{490} determination.

c. After completing the Na-fluorescein penetration steps, the corneas should be fixed in an appropriate fixative (e.g., 10% neutral buffered formalin) at room temperature for at least 24 hours, so that the tissues are available if histology is necessary or requested at a later time. It is important that the corneas not be allowed to dry between transfer from the holders and fixation (submersion in the fixative).

d. If using a microplate reader to measure optical density, transfer 360 µL of the medium from each sample tube into its designated well on a 96-well plate. The standard plate map provides two wells for each cornea. The first well receives an undiluted sample from each cornea tested. When all of the media samples have been transferred onto the plate, measure and record their OD_{490}. Any OD_{490} value (of a control or test substance sample) that is 1.500 or greater must be diluted to bring the OD_{490} into the acceptable range. A dilution of 1:5 is generally sufficient but higher dilutions may be required. Prepare the dilution from the original sample of medium and transfer 360 µL into the second well designated for that cornea. Reread the plate and record the data from both

the undiluted and diluted OD_{490} values. Use the values from this second reading in all calculations. The OD_{490} values of less than 1.500 will be used in the permeability calculation.

Note: The linear range of absorbance of different microplate readers can vary. Thus, each laboratory must determine the upper limit of absorbance (in the linear range) for the microplate reader used in its facility.

e. If using a UV/VIS spectrophotometer to measure optical density, adjust the spectrophotometer to read at OD_{490}, and zero the spectrophotometer on a sample of complete MEM. Prior to reading samples from the BCOP assay, prepare and read two quality control samples of Na-fluorescein solution to ensure the Na-fluorescein calibration curve (see note below) conducted for the spectrophotometer is still acceptable. If the average of the quality control samples does not fall within the accepted range of the Na-fluorescein calibration curve, then prepare a Na-fluorescein calibration curve prior to running samples from the BCOP assay. If the average of the quality control samples falls within the accepted range of the calibration curve, then proceed to read samples from the BCOP assay. Transfer an aliquot of the mixed medium from the posterior chamber of the BCOP holder into a cuvette, then take and record an absorbance reading using the spectrophotometer. Any solutions giving an OD_{490} beyond the linear range of the spectrophotometer must be diluted in complete MEM, and another reading taken, repeating these steps until the OD_{490} is within the linear range of the spectrophotometer. Repeat these procedures for each sample from the BCOP assay, rinsing the cuvette(s) thoroughly between each sample, until all samples have been read and results recorded.

Note: If conducting this assay for the first time, a calibration curve for the spectrophotometer must be performed, using a series of dilutions of Na-fluorescein solution in complete MEM. A calibration curve should be prepared and used to determine the linear range of the spectrophotometer and thus determine the upper limit of absorbance.

6.8 Histopathology

A histopathological evaluation of the corneal tissue might be useful when the standard BCOP endpoints (i.e., corneal opacity and permeability) produce borderline results. A standardized scoring scheme using the formal language of pathology to describe any effects should be used.

6.9 Maintenance of the Corneal Holders

Following completion of the assay, clean the disassembled parts of each holder as follows:

a. Soak the posterior and anterior chambers in a solution of warm tap water and a dime-size or greater amount of Liquinox (or equivalent).

b. Soak the chamber plugs, O-rings, and handle screws in 70% ethanol. Rinse the chamber plugs, O-rings, and handle screws thoroughly in hot tap water, and air dry prior to reassembling the chambers.

c. Clean the interior and exterior surfaces of each pre-soaked posterior and anterior chamber by using a scrubbing sponge. Rinse each posterior and anterior chamber thoroughly in warm tap water and air dry prior to reassembling the chambers.

d. Match up each numbered posterior chamber with its corresponding anterior chamber; insert an O-ring into the appropriate place; attach a chamber handle screw to the anterior chamber; and finally insert the chamber screws into the anterior chamber.

7.0 Evaluation of Test Results

Results from the two test method endpoints, opacity and permeability, should be combined in an empirically derived formula that generates an *in vitro* irritancy score for each test substance.

7.1 Opacity

a. Calculate the change in opacity for each individual cornea (including the negative control) by subtracting the initial opacity reading from the final post-treatment opacity reading. Then calculate the average change in opacity for the negative control corneas.

b. Calculate a corrected opacity value for each treated cornea, positive control, and solvent/vehicle control (if applicable) by subtracting the average change in opacity of the negative control corneas from the change in opacity of each treated, positive control, or solvent/vehicle control cornea.

c. Calculate the mean opacity value of each treatment group by averaging the corrected opacity values of the treated corneas for each treatment group.

7.2 Permeability

<u>Microplate Reader Method</u>

a. Calculate the mean OD_{490} for the blank wells (plate blanks). Subtract the mean blank OD_{490} from the raw OD_{490} of each well (blank corrected OD_{490}).

b. If a dilution has been performed, correct the OD_{490} for the plate blank before the dilution factor is applied to the reading. Multiply each blank corrected OD_{490} by the dilution factor (e.g., a factor of 5 for a 1:5 dilution).

c. Calculate the final corrected OD_{490} value for each cornea by subtracting the mean OD_{490} value for the negative control corneas from the OD_{490} value of each treated cornea.

   Final Corrected OD_{490} = (raw OD_{490} – mean blank OD_{490}) -mean blank corrected negative control OD_{490}

d. Calculate the mean OD_{490} value for each treatment group by averaging the final corrected OD_{490} values of the treated corneas for a particular treatment group.

<u>UV/VIS Spectrophotometer Method</u>

a. Calculate the corrected OD_{490} value of each treated, positive control, or solvent/vehicle control cornea by subtracting the average value of the negative control corneas from the original OD_{490} value for each cornea.

   Final Corrected OD_{490} = raw OD_{490} -mean blank corrected negative control OD_{490}

b. Calculate the mean OD_{490} value for each treatment group by averaging the final corrected OD_{490} values of the treated corneas for a particular treatment group.

7.3 *In Vitro* Irritancy Score

Use the mean opacity and mean permeability values (OD_{490}) for each treatment group to calculate an *in vitro* score for each treatment group:

In Vitro Irritancy Score = mean opacity value + (15 x mean OD_{490} value)

Additionally, the opacity and permeability values should be evaluated independently to determine whether a test substance induced irritation through only one of the two endpoints.

8.0 Criteria for an Acceptable Test

A test is acceptable if the positive control gives an *in vitro* irritancy score that falls within two SDs of the current historical mean, which is to be updated at least every three months. In the BCOP, 100% ethanol induces a moderate to severe response (*in vitro* score = 39.9 - 65.4 at IIVS [n = 632]; mean = 52.7, standard deviation [SD] = 6.4), while 20% (w/v) imidazole induces a severe response (*in vitro* score = 69.7 - 136.2 at IIVS [n=125]; mean = 103, SD = 16.6). The negative or solvent/vehicle control responses should result in opacity and permeability values that are less than the established upper limits for background opacity and permeability values for bovine corneas treated with the respective negative or solvent/vehicle control.

9.0 Data Interpretation

The following classification system was established by Sina et al. (1995) based on studies with pharmaceutical intermediates exposed for 10 minutes (liquids) or 4 hours (solids).

In Vitro Score: 55.1 and above = severe irritant

While this classification system provides a good initial guide to interpretation of these *in vitro* data, these specific ranges may not be applicable to all classes of substances. For example, the Sina et al. (1995) scoring scale is not appropriate for anionic and nonionic surfactants since they produce appreciable permeability while inducing little direct opacity.

For these and other substances that produce significant permeability with minimal opacity, it is recommended that permeability values > 0.600 be considered severe. Benchmark substances are recommended for assaying the responses of test substances of different product or chemical classes. Histological evaluation of the corneas may be instrumental in identifying additional changes (e.g., peroxide-induced stromal damage).

10.0 Study Report

The test report should include the following information, if relevant to the conduct of the study:

Test and Control Substances

- Chemical name(s) such as the structural name used by the Chemical Abstracts Service (CAS), followed by other names, if known
- The CAS Registry Number (RN), if known
- Purity and composition of the substance or preparation (in percentage[s] by weight), to the extent this information is available
- Physicochemical properties such as physical state, volatility, pH, stability, chemical class, water solubility relevant to the conduct of the study
- Treatment of the test/control substances prior to testing, if applicable (e.g., warming, grinding)
- Stability, if known

Information Concerning the Sponsor and the Test Facility

- Name and address of the sponsor, test facility, and study director
- Identification of the source of the eyes (i.e., the facility from which they were collected)

- Storage and transport conditions of eyes (e.g., date and time of eye collection, time interval prior to initiating testing, transport media and temperature conditions, any antibiotics used)
- If available, specific characteristics of the animals from which the eyes were collected (e.g., age, sex, strain, weight of the donor animal)

Justification of the Test Method and Protocol Used

Test Method Integrity

- The procedure used to ensure the integrity (i.e., accuracy and reliability) of the test method over time (e.g., periodic testing of proficiency substances, use of historical negative and positive control data)

Criteria for an Acceptable Test

- Acceptable concurrent positive and negative control ranges based on historical data
- If applicable, acceptable concurrent benchmark control ranges based on historical data

Test Conditions

- Description of test system used
- Type of corneal holder used
- Calibration information for devices used for measuring opacity and permeability (e.g., opacitometer and spectrophotometer)
- Information on the bovine corneas used, including statements regarding their quality
- Details of test procedure used
- Test substance concentration(s) used
- Description of any modifications of the test procedure
- Reference to historical data of the model (e.g., negative and positive controls, proficiency substances, benchmark substances)
- Description of evaluation criteria used

Results

- Tabulation of data from individual test samples (e.g., opacity and OD_{490} values and calculated *in vitro* irritancy score for the test substance and the positive, negative, and benchmark controls [if included], reported in tabular form, including data from replicate repeat experiments as appropriate, and means ± the standard deviation for each experiment)
- Description of other effects observed

Discussion of the Results

Conclusion

A Quality Assurance Statement for Good Laboratory Practice (GLP)-Compliant Studies

- This statement indicates all inspections made during the study, and the dates any results were reported to the study director. This statement also serves to confirm that the final report reflects the raw data.

If GLP-compliant studies are performed, then additional reporting requirements provided in the relevant guidelines (e.g., OECD 1998; EPA 2003b, 2003c; FDA 2003) should be followed.

11.0 References

Doughty MJ, Petrou S, and Macmillan H. 1995. Anatomy and morphology of the cornea of bovine eyes from a slaughterhouse. Can J Zool 73: 2159-2165.

EPA. 2003a. Label Review Manual: 3rd ed. EPA737-B-96-001. Washington, DC:U.S. Environmental Protection Agency.

EPA. 2003b. Good Laboratory Practice Standards. 40 CFR 792.

EPA. 2003c. Good Laboratory Practice Standards. 40 CFR 160.

EU. 2001. Commission Directive 2001/59/EC of 6 August 2001 adapting to technical progress for the 28th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. Official Journal of the European Communities L255:1333.

FDA. 2003. Good Laboratory Practice for Nonclinical Laboratory Studies. 21 CFR 58.

Gautheron P, Dukic M, Alix D, and Sina JF. 1992. Bovine corneal opacity and permeability test: An *in vitro* assay of ocular irritancy. Fundam Appl Toxicol 18:442–449.

ICCVAM. 2006a. Background Review Document: Current Status of *In Vitro* Test Methods for Identifying Ocular Corrosives and Severe Irritants: Bovine Corneal Opacity and Permeability NIH Publication No. 06-4512. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih/gov/docs/ocutox_docs/ocubrd/bcop/bcopbrd.pdf.

ICCVAM. 2006b. ICCVAM Test Method Evaluation Report: *In Vitro* Ocular Toxicity Test Methods for Identifying Severe Irritants and Corrosives. NIH Publication No. 07-4517. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/docs/ocutox_docs/OTeval/OTevalrpt.pdf.

INVITTOX 1999. Protocol 124: Bovine Corneal Opacity and Permeability Assay – SOP of Microbiological Associates Ltd. Available: http://ecvam-sis.jrc.it/.

OECD. 1998. OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring Number 1: OECD principles on Good Laboratory Practice (as revised in 1997). ENV/MC/CHEM(98)17. Paris:OECD. Available: http://www.oecd.org.

Sina JF, Galer DM, Sussman RG, Gautheron PD, Sargent EV, Leong B, et al. 1995. A collaborative evaluation of seven alternatives to the Draize eye irritation test using pharmaceutical intermediates. Fundam Appl Toxicol 26:20–31.

Ubels JL, Ditlev JA, Clusing DP, Casterton PL. 2004. Corneal permeability in a redesigned corneal holder for the bovine cornea opacity and permeability assay. Toxicol In Vitro 18:853–857.

Ubels JL, Paauw JD, Casterton PL, Kool DJ. 2002. A redesigned corneal holder for the bovine cornea opacity and permeability assay that maintains normal corneal morphology. Toxicol In Vitro 16:621–628.

UN. 2007. Globally Harmonized System of Classification and Labelling of Chemicals (GHS), 2nd rev ed, New York: United Nations. [Available: http://www.unece.org/trans/danger/publi/ghs/ghs_rev02/02files_e.html.]

This page intentionally left blank

Appendix B2

ICCVAM-Recommended Protocol for Future Studies Using the Cytosensor Microphysiometer (CM) Test Method

This page intentionally left blank

PREFACE

This proposed protocol for ocular toxicity is based primarily on information obtained in INVITTOX Protocol 102 derived from the standard operation procedure used in the Home Office UK/EEC Validation Study for Alternatives to the Draize Test. The information contained within INVITTOX 102 was modified based upon the COLIPA protocol (Brantom et al., 1997; Harbell et al., 1999). Future studies using the CM test method could include further characterization of the usefulness and limitations of the CM test method in a weight-of-evidence approach for regulatory decision-making. Users should be aware that the proposed test method protocol could be revised based on any additional optimization and/or validation studies that are conducted in the future. ICCVAM recommends that test method users consult the NICEATM-ICCVAM website (http://iccvam.niehs.nih.gov/) to ensure use of the most current test method protocol.

1.0 Purpose and Applicability

The purpose of this study is to compare the ocular toxicity of the test material as predicted using the CM method with historical rabbit Draize eye data. The CM method evaluates the potential ocular toxicity by measuring the test material induced reduction in the metabolic rate in treated cultures of L929 cells. Change in metabolic rate is measured indirectly as a function of changes in extracellular acidification rate. The dose that induces a 50% decrease in metabolic rate is the end point of the assay.

The focus of this protocol is on the use of the CM test method for the detection of ocular corrosives and severe irritants and substances not labeled as irritants as defined by the U.S. Environmental Protection Agency (EPA; EPA 2003a), the European Union (EU; EU 2001), and United Nations Globally Harmonized System (GHS) of Classification and Labelling of Chemicals (UN 2007). Mild/moderate ocular irritants have been tested using this protocol; however, the CM test method is not currently considered to be adequately validated for these classes of ocular irritancy as defined by EPA (2003a), EU (2001), and GHS (UN 2007).

2.0 Safety and Operating Precautions

All procedures with L929 cells should follow the institution's applicable regulations and procedures for handling human or animal substances, which include, but are not limited to, tissues and tissue fluids. Universal laboratory precautions are recommended, including the use of laboratory coats, eye protection, and gloves. If available, additional precautions required for specific study substances should be identified in the Material Safety Data Sheet for that substance.

3.0 Materials, Equipment, and Supplies

3.1 Equipment and Supplies

- Aspirator
- Balance
- Beakers, disposable
- Capsules, eight with L-929 cells grown to be <80% confluent at time of use (confluent monolayer could interfere with accurate CM readings) in DMEM. To prepare these, load 5-6 x 10^5 cells about 18 hr prior to use and incubate in complete DMEM with 1% calf serum under standard culture conditions.
- Cell culture equipment for preparation of cells
- Cytosensor System with eight sterilized chambers, set up in the injection loop mode - Molecular Devices Corporation, Menlo Park, California, USA

- Cytosoft and the following Cytosoft protocols for toxicity testing:
  - Tox Maintenance (ii) Routine Tox 003 (4x2) (both supplied by MDC)
  - A statistics program capable of MRD_{50}
- Pipettors, rack, etc., for preparation of dilutions
- Refrigerator
- Statistical program for calculation of MRD_{50}
- Tubes, 15 ml, for preparation of dilutions (4 dilutions per test sample).
- Tube racks
- Syringes, 4 x 5 ml and a 30 ml
- Water bath

3.2 Media and Reagents

- Assay Medium: DMEM complete with 1% Fetal Bovine Serum, 5.0 μg/ml gentamicin, 2.0 mM L-glutamine, and 1.0 mM sodium pyruvate.
- Growth Medium: Dulbecco's modified Eagle's medium (DMEM) (1mg/ml glucose) complete with 10% Fetal Bovine Serum, 2.0 mM L-glutamine, and 1.0 mM sodium pyruvate.
- Positive Control: Sodium lauryl sulfate (SLS) 10% in water (stock).
- Treatment medium: Serum-free, Sodium Bicarbonate-free, DMEM with 5.0 μg/ml gentamicin, 2.0 mM L-glutamine, and additional NaCl for consistent osmolarity (MDMEM). 11.1 ml of 4 M NaCl is required per liter.
- Trypsin, 0.05% in Ca^{+2} and Mg^{+2}-free Hank's Balanced Salt Solution.

4.0 Test Substance Preparation

The test article will be dissolved in MDMEM. It is essential that the test material be in a single-phase solution in the highest dose used (300 mg/mL) to prepare the subsequent dilutions. If the substance cannot form a single phase solution/suspension at a concentration of 33.3 mg/mL, the test sample cannot be tested by the CM using standard techniques.

The stability of the test article under the actual experimental conditions will not be determined by the testing laboratory.

5.0 Controls

5.1 Negative Control

The baseline acidification rate will serve as the internal negative control for each cell culture. Baseline rates will fall between 50 and 150 microvolts/sec after a stabilization period of at least 15 minutes. Replace the cell-containing insert in a chamber that fails to achieve these ranges.

5.2 Solvent/Vehicle Control

Untreated controls are recommended when solvents/vehicles other than 0.9% sodium chloride or distilled water are used to dissolve test substances, in order to demonstrate that the solvent/vehicle is not interfering with the test system.

5.3 Positive Control

When the 8-channel Cytosensor is used, a positive control assay will be performed with each definitive trial of the assay. When the 4-channel machine is used, a concurrent positive control trial

will be performed with at least one of the definitive trials for each test material. The positive control substance is SLS prepared from a 10% stock in water.

5.4 Benchmark Substances (if appropriate)

Benchmark substances are useful for evaluating the ocular irritancy potential of unknown chemicals of a specific chemical or product class, or for evaluating the relative irritancy potential of an ocular irritant within a specific range of irritant responses. Appropriate benchmark substances should have the following properties:

- A consistent and reliable source(s)
- Structural and functional similarity to the class of the substance being tested
- Known physical/chemical characteristics
- Supporting data on known effects in the *in vivo* rabbit eye test
- Known potency in the range of the desired response

6.0 Experimental Design

6.1 Filling the Workstations with Medium

Put 8 x 50 ml tubes, each having at least 20 ml of MDMEM on the Cytosensor and fill the injection loops with MDMEM, using a 30 ml syringe. Using the "Front Panel" controls, set the flow rate to 90-100% to fill the lines, and then set the flow rate back to idle (5%).

6.2 Checking out the Equipment

Empty Sterilant from the sensor chambers, wash them by repeated filling with, and aspiration of, distilled water, and then add about 2 ml of low-buffer DMEM to each chamber. Put them on the Cytosensor. Set flow rate to High (90-100% of max) and clear obvious bubbles. Run Cytosoft default protocol ("New") to see that system sets up and the background rate in the absence of cells settles within 10 minutes to between +5 and -5 microvolts/sec. This gives the opportunity to attend to any equipment problems before starting to use cells.

6.3 Checking out the Cells

Exit "New" protocol and set flow rate to Normal (approx. 50%) using "Front Panel" controls. To at least 8 cell-containing cell capsules in a culture tray containing Low-Buffered DMEM, add spacers and inserts as described in the Manual. Move the tray to the Cytosensor and use forceps to transfer the completed capsules to the sensor chambers, lifting the gantries and raising the plungers one set at a time. When all the capsules are in place, set the flow rate to High and clear obvious bubbles again.

6.4 Cell Culture Maintenance and Preparation of the Capsule Cups

Stock cultures of L929 cells will be maintained and passaged in Growth Medium and incubated at $37 \pm 1^{o}C$ and $5 \pm 1\%$ CO_2 in air. L929 cells will be seeded onto capsule cups at approximately 6.0×10^5 cells per capsule cup in Seeding Medium as described below.

Flasks of L929 cells to be passaged or seeded are selected at or near confluency. The size of flasks used will depend on the number of cells needed. The Growth Medium is decanted and the cell sheet washed twice with approximately 10 mL of PBS for each $75cm^2$ of growth surface. The cells are trypsinized with approximately 3 mL of 0.05% trypsin (for each $75cm^2$ of growth surface) for 15 to 30 seconds. The trypsin solution is aspirated and the cells are incubated at room temperature for approximately 2 to 5 minutes, until the cells begin to round. The cells are dislodged by tapping the flask, which contains approximately 5mL of Seeding Medium for each $75cm^2$ of growth surface. The

cells are triturated using a pipet in order to break up clumps and are transferred by pipet to a conical centrifuge tube. If more than one flask is used, the contents of each are pooled. Cell counts are performed as required. The L929 cells will be seeded with approximately 6.0 x 10^5 cells per each capsule cup (0.5 mL of a 1.2 x 10^6 cell suspension) with 1.5 mL of Seeding Medium added to each outside well. The plate will be incubated at 37 ± 1°C and 5 ± 1% CO_2 in air for 16 to 32 hours. Prior to the start of the assay, the medium in capsule cups will be switched to Low-Buffered DMEM and a spacer will be added to each capsule cup and gently tapped down to the bottom. The cell capsules will be placed into the sensor chambers and exposed to Low-Buffered DMEM at 37 ± 1°C.

For routine passaging, the stock cultures are trypsinized as described above, but are dislodged and resuspended using warm (approximately 37°C) Growth Medium, seeded into a culture flask(s), and returned to the humidified incubator maintained at 37 ± 1°C and 5 ± 1% CO_2 in air.

6.5 Dose Range Finding Assay

A dose range finding assay will be performed to establish an appropriate test article dose range for the definitive CM assay. Dosing solutions will be prepared by serial three-fold dilutions (producing the same concentrations suggested in the following table) in sterile, Low-Buffered DMEM that has been allowed to equilibrate to room temperature.

IMPORTANT: Do not attempt to use preparations that separate into more than one phase in the Cytosensor. Similarly, do not attempt to use such preparations to make dilutions. At the discretion of the Study Director, a suspension that maintains a single phase may be assayed and used to prepare further dilutions.

If the sample does not go into a single phase with the medium at 10.0 mg/mL (maintaining a ratio of 100 mg/10 mL), prepare dilutions 2 or 3 as required. If a single-phase test article/medium mixture is not achieved, the Study Director and Sponsor are to be consulted.

Dilution #	Concentration
1	10 mg/mL
2	3.33 mg/mL
3	1.11 mg/mL
4	0.370 mg/mL
5	0.123 mg/mL
6	0.0412 mg/mL
7	0.0137 mg/mL

The test article will be evaluated by exposure to L929 cells contained in sensor chambers. After the baseline data points have been taken, the exposure cycle will begin with the lowest test article concentration. From these baseline data points, the spreadsheet will compute the mean baseline value used in the MRD_{50} calculation. Each exposure cycle will take 20 minutes.

The maximum solvent concentration (other than Low-Buffered DMEM) will be 10% unless otherwise specified.

There will be three phases in the exposure cycle, with the following parameters selected within the CM software (Cytosoft): First, a test article concentration will be introduced into the sensor chamber for 13 minutes and 30 seconds. The nominal rate of flow will be 100 µL per minute for the first minute, and 20 µL per minute for the next 12 minutes and 30 seconds. The second phase will be the washout phase, which will be 6 minutes at a nominal rate of 100 µL per minute. The test article will

be washed out of the sensor chamber during this phase. Finally, the third phase will be the measurement of the acidification rate. For 25 seconds, there will be no flow and the rate of pH change will be measured.

The exposure cycle will repeat with increasing test article concentrations until either the highest test article concentration is reached or until the MRD_{50} value has been surpassed. Each test article concentration will be tested on a single set of cells. Positive control materials and solvent controls (for solvents other than Low-Buffered DMEM) will be tested in the same fashion. If possible, an MRD_{50} value will be calculated from the dose range finding assay.

The test article doses for the definitive assay will be chosen so that generally seven doses (spaced as three-fold dilutions) will be available for the determination of the MRD_{50}. Generally, three concentrations will be chosen to result in expected survivals lower than 50%, one concentration will be chosen to result in an expected survival of approximately 50%, and three or more concentrations will be chosen to result in expected survivals greater than 50%. If a test article fails to cause 50% toxicity in the dose range finding CM assay, the maximum dose will generally be 270 mg/mL, or less based on its solubility/workability.

6.6 Definitive Assay

The definitive assay will be performed in the same manner as the dose range finding assay, with the exception that if the MRD_{50} value from the dose range finding assay is >10 mg/mL, higher doses of test article will be prepared and tested in the definitive assay. At least seven doses, spaced at three-fold dilution intervals, up to a maximum of 270 mg/mL will be prepared. The determination of the final MRD_{50} will be based upon the results of at least two definitive assays and will generally also include the results of the dose range finding assay, if an MRD_{50} could be determined. The results from additional definitive assays may also be incorporated into the calculation of the final MRD_{50}.

7.0 Evaluation of Test Results

The acidification rates that occur after exposure to each test article concentration are calculated by the CM software (Cytosoft) and compared to the mean acidification rate (base acidification rate) of the same cells prior to exposure to a test material. The percent of control acidification rate will be determined by comparing the dose response acidification rate to the base acidification rate. The dose response curve will be plotted with the percent of control acidification rates on the ordinate and the test article concentration on the abscissa. The concentration of the test material that results in a fifty percent reduction in acidification is interpolated from the curve and referred to as the MRD_{50}. These calculations can be performed using the Excel spreadsheet program provided for this study.

8.0 Criteria for an Acceptable Test

Assay acceptance criteria are normally based on the performance of the positive control. The CM assay would be accepted if the positive control MRD_{50} fell within 2 standard deviations of the historical range. The acceptable range for SLS will be provided by the lead laboratory. The positive control assay will not be performed with each trial on the 4-channel machine. Therefore, acceptance of those trials, lacking a positive control, will be based on judgment of the study director.

9.0 Data Interpretation

Interpretation of MRD_{50} values is done according to the decision criteria provided in Background Review Document: Existing Methods for Eye Irritation Testing: Silicon Microphysiometer and CM (ECVAM 2008), as follows:

For the EU system (EU 2001) the proposed PM is

	MRD_{50}
R41	<2 mg/mL
R36	<10 mg/mL; >2 mg/mL
Not classified	>10 mg/mL

For the GHS system (UN 2007) the proposed PM is

	MRD_{50}
1	<2 mg/mL
2A or 2B	<10 mg/mL; >2 mg/mL
No Label	>10 mg/mL

For the EPA system (EPA 2003a) the proposed PM is

	MRD_{50}
1	<2 mg/mL
III	<80 mg/mL; >2 mg/mL
Not classified	>80 mg/mL

10.0 Study Report

The test report should include the following information, if relevant to the conduct of the study:

Test and Control Substances

- Chemical name(s) such as the structural name used by the Chemical Abstracts Service (CAS), followed by other names, if known
- The CAS Registry Number (RN), if known
- Purity and composition of the substance or preparation (in percentage(s) by weight), to the extent this information is available
- Physicochemical properties such as physical state, volatility, pH, stability, chemical class, water solubility relevant to the conduct of the study
- Treatment of the test/control substances prior to testing, if applicable (e.g., warming, grinding)
- Stability, if known

Information Concerning the Sponsor and the Test Facility

- Name and address of the sponsor
- Name and address of the test facility
- Name and address of the Study Director

Justification of the Test Method and Protocol Used

Test Method Integrity

- The procedure used to ensure the integrity (i.e., accuracy and reliability) of the test method over time (e.g., periodic testing of proficiency substances, use of historical negative and positive control data)

Criteria for an Acceptable Test

- Acceptable concurrent negative control ranges based on historical data
- Acceptable concurrent positive control ranges based on historical data
- If applicable, acceptable concurrent benchmark control ranges based on historical data

Test Conditions

- Description of test system used
- Calibration information for measuring device used
- Details of test procedure used
- Test concentration(s) used
- Description of any modifications of the test procedure
- Reference to historical data of the model (e.g., negative and positive controls, proficiency substances, benchmark substances)
- Description of evaluation criteria used

Results

- Tabulation of data from individual test samples

Description of Other Effects Observed

Discussion of the Results

Conclusion

A Quality Assurance Statement for Good Laboratory Practice (GLP)-Compliant Studies

- This statement indicates all inspections made during the study, and the dates any results were reported to the Study Director. This statement also serves to confirm that the final report reflects the raw data.

If GLP-compliant studies are performed, then additional reporting requirements provided in the relevant guidelines (e.g., OECD 1998; EPA 2003b, 2003c; FDA 2003) should be followed.

11.0 References

Brantom PG, Bruner LH, Chamberlain M, De Silva O, Dupuis J, Earl LK, et al. (1997). A summary report of the COLIPA international validation study on alternatives to the Draize rabbit eye irritation test. Toxicol In Vitro 11:141–179.

Cytosensor Microphysiometer System User's Manual. Sunnyvale, CA:Molecular Devices.

ECVAM. 2008. Background Review Document: Existing Methods for Eye Irritation Testing: Silicon Microphysiometer and Cytosensor Microphysiometer. Contract No: CCR.IHCP.C431305.X0. Ispra, Italy:Institute for Health and Consumer Protection.

EPA. 2003a. Label Review Manual: 3rd ed. EPA737-B-96-001. Washington, DC:U.S. Environmental Protection Agency.

EPA. 2003b. Good Laboratory Practice Standards. 40 CFR 792.

EPA. 2003c. Good Laboratory Practice Standards. 40 CFR 160.

EU. 2001. Commission Directive 2001/59/EC of 6 August 2001 adapting to technical progress for the 28th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. Official Journal of the European Communities L255: 1–333.

FDA. 2003. Good Laboratory Practice for Nonclinical Laboratory Studies. 21 CFR 58.

Harbell JW, Osborne GJ, Carr GJ, Peterson A. (1999). Assessment of the Cytosensor Microphysiometer assay in the COLIPA in vitro eye irritation validation study. Toxicol In Vitro 13:313–323.

OECD. 1998. OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring Number 1: OECD Principles on Good Laboratory Practice [as revised in 1997]. ENV/MC/CHEM(98)17. Paris: Organisation for Economic Co-operation and Development. Available: http://www.oecd.org.

UN. 2007. Globally Harmonized System of Classification and Labelling of Chemicals (GHS). 2nd rev ed. New York:United Nations Publications. Available: [http://www.unece.org/trans/danger/publi/ghs/ghs_rev02/02files_e.html].

Appendix B3

ICCVAM-Recommended Protocol for Future Studies Using the Hen's Egg Test – Chorioallantoic Membrane (HET-CAM) Test Method

ICCVAM recommends this HET-CAM test method protocol for nonregulatory, validation, or optimization studies to facilitate collection of consistent data and expand the available database. Exceptions and/or changes to the test method protocol should be accompanied by a scientific rationale.

This page intentionally left blank

PREFACE

The protocol was adapted from the protocol previously described by Spielmann and Liebsch (INVITTOX 1992). Examples of the use of this protocol can be found in Luepke (1985), Balls et al. (1995), Gilleron et al. (1996, 1997), and Spielmann et al. (1996). Future studies using the HET-CAM test method could include further characterization of the usefulness and limitations of the HET-CAM test method in a weight-of-evidence approach for regulatory decision-making. Users should be aware that the proposed test method protocol could be revised based on any additional optimization and/or validation studies that are conducted in the future. ICCVAM recommends that test method users consult the NICEATM-ICCVAM website (http://iccvam.niehs.nih.gov/) to ensure use of the most current test method protocol.

1.0 Purpose and Applicability

The purpose of this protocol is to describe the components and procedures used to evaluate the potential ocular irritancy of a test substance as measured by its ability to induce toxicity in the chorioallantoic membrane of a chicken. Effects are measured by the onset of (1) hemorrhage; (2) coagulation; and (3) vessel lysis. These assessments are considered individually and then combined to derive a score, which is used to classify the irritancy level of the test substance.

The focus of this protocol is on the use of the HET-CAM test method for the detection of ocular corrosives and severe irritants, as defined by the U.S. Environmental Protection Agency (EPA 2003a), European Union (EU; EU 2001), and United Nations Globally Harmonized System (GHS) of Classification and Labelling of Chemicals (UN 2007). However, the HET-CAM test method is not currently considered to be adequately validated for classification of ocular irritancy as defined by EPA (2003a), EU (2001), and GHS (UN 2007).

This HET-CAM test method protocol has been modified from a generic description of the Irritation Score (IS) analysis method to include a more detailed IS(A) analysis method to be used for prospective studies. However, a description of the IS(B) analysis method, which was described in 2006 (ICCVAM 2006) is included for retrospective analyses, where IS(B) analysis method data could be converted to fixed time points similar to those used for the IS(A) analysis method described in **Section 7.0**.

ICCVAM recommends this HET-CAM test method protocol for nonregulatory, validation, or optimization studies to facilitate collection of consistent data and expand the available database. Exceptions and/or changes to the test method protocol should be accompanied by a scientific rationale.

2.0 Safety and Operating Precautions

All procedures with chicken eggs should follow the institution's applicable regulations and procedures for handling of human or animal materials, which include, but are not limited to, tissues and tissue fluids. Universal laboratory precautions are recommended, including the use of laboratory coats, eye protection, and gloves. If available, additional precautions required for specific study substances should be identified in the Material Safety Data Sheet for that substance.

3.0 Materials, Equipment, and Supplies

3.1 Source of Chicken Eggs

Fertile White Leghorn chicken eggs should be obtained from commercial sources. Fresh (not older than seven days), fertile, clean eggs weighing between 50 and 60 grams should be used. Eggs should

be candled prior to use and nonviable or defective eggs should be discarded. Excessively misshapen eggs or eggs with cracked or thin shells should not be used. Transport of eggs should occur under conditions that will not affect embryo viability or development.

3.2 Equipment and Supplies

- Candling light
- Deionized/Distilled Water
- Dentist's rotating saw blade
- Incubator with an automatic rotating device
- Micropipette(s) and disposable tips appropriate for recommended volumes
- Mortar and pestle (or comparable grinding tools for test substances)
- Stop clock or electronic chronometer
- Standard general biological laboratory equipment and supplies (e.g., microcentrifuge tubes for measurement of substance volume), as needed
- Tapered forceps
- Volumetric flasks

3.3 Solutions

The manufacturer's recommendations should be followed with regard to storage temperature and shelf life of stock solutions. Solutions should be prepared volumetrically.

- 0.9% (w/v) sodium chloride (NaCl) in deionized/distilled water
- 1% (w/v) sodium dodecyl sulfate (SDS) in deionized/distilled water
- 0.1 N sodium hydroxide (NaOH) in deionized/distilled water

4.0 Test Substance Preparation

All test substances should be evaluated undiluted unless dilution is justified. If dilution is justified, then 0.9% NaCl or olive oil should be used as the diluent, depending on substance solubility. Use of a different solvent should be justified. Dilutions should be prepared on the same day as the test.

Paste, particulate, or granular test substances or formulations should be evaluated without dilution. Solid test substances should be ground to a fine dust to obtain a volume of 0.3 mL after gentle compaction of the particulates in a measuring container (e.g., microcentrifuge tube).

5.0 Controls

5.1 Negative Control

A 0.9% NaCl negative control should be included in each experiment in order to provide a baseline for the assay endpoints and to ensure that the assay conditions do not inappropriately result in an irritant response.

5.2 Solvent Control (if appropriate)

If the test substance is diluted in olive oil, then this solvent should be included as a control substance in order to provide a baseline for the assay endpoints and to ensure that the assay conditions do not inappropriately result in an irritant response. If a solvent other than 0.9% NaCl or olive oil is used, than both the solvent and 0.9% NaCl should be included as controls to ensure that the alternative solvent does not result in an irritant response.

5.3 Positive Control

A known ocular irritant should be included in each experiment to verify that an appropriate response is induced. If the HET-CAM assay is being used only to identify corrosive or severe irritants, then the positive control should be a substance (e.g., 1% SDS, NaOH) that induces a severe response *in vivo* as well as in HET-CAM. However, to ensure that variability in the positive control response across time can be assessed, the magnitude of the severe response should not be excessive. The selection of positive control test substances should be based on the availability of high quality *in vivo* data.

5.4 Benchmark Control (if appropriate)

Benchmark controls may be useful in demonstrating that the test method is functioning properly for detecting the ocular irritancy potential of chemicals of a specific chemical class or a specific range of responses, or for evaluating the relative irritancy potential of an ocular irritant. Appropriate benchmark controls should have the following properties:

- A consistent and reliable source(s)
- Structural and functional similarity to the class of the substance being tested
- Known physical/chemical characteristics
- Supporting data on known effects in the *in vivo* rabbit eye test
- Known potency in the range of the desired response

6.0 Experimental Design

6.1 Treatment Groups

Use at least three eggs per group (negative and positive controls, test substance, and, if included, benchmark and solvent controls). To the extent possible, eggs from the same hen should be randomized among treatment groups.

6.2 CAM Preparation

a. Select fresh (not older than 7 days), clean, fertile 50-60 g White Leghorn chicken eggs. Candle the eggs and discard any eggs that are nonviable or defective. Excessively misshapen eggs or eggs with cracked or thin shells should not be used. Shaking, unnecessary tilting, knocking, and all other mechanical irritation of the eggs should be avoided when preparing.

b. Place eggs in an incubator with a rotating tray. Incubate eggs at 38.3 ± 0.2°C and 58 ± 2% relative humidity when incubating in a still-air incubator or at 37.8 ± 0.3ºC and 58 ± 2% relative humidity when incubating in a forced-air incubator. Hand rotate eggs five times per day until day 8.

c. Candle the eggs on incubation day 8 and remove any nonviable or defective eggs. Eggs are returned to the incubator (without hand rotation) with the large end of the eggs upwards for an additional day.

d. Remove eggs from the incubator on day 9 for use in the assay. Candle eggs and discard any nonviable or defective eggs.

e. Mark the air cell of the egg. Cut the section marked as the air cell with a rotating dentist saw blade and then pare it off. Care should be taken when removing the eggshell to ensure that the inner membrane is not injured.

f. Moisten the inner membrane with 0.9% NaCl. A disposable glass pipette can be used to apply the solution. Place the egg into the incubator for a maximum of 30 minutes.

g. Remove the egg from the incubator, prior to its use in the assay, and decant the 0.9% NaCl solution. Carefully remove the inner membrane with forceps, ensuring that the inner membrane is not injured.

6.3 Treatment of Eggs with Test Substances

Depending on the physical form of the test substance, the following form-specific application protocols should be followed.

6.3.1 Liquid or diluted test substances or formulations

Apply 0.3 mL of liquid substances or diluted substances directly onto the CAM surface.

6.3.2 Solid, particulate, or granular test substances or formulations

Apply 0.3 mL of solid, particulate, or granular substances (which have been ground to a fine dust) directly onto the CAM, ensuring that at least 50 % of the CAM surface area is covered. In cases where the total weight of the test substance at this volume is greater that 0.3 g, 0.3 g of the solid, particulate, or granular test substance should be used. In either case, the weight of the test substance should be recorded.

6.3.3 Paste test substances or formulations

Apply 0.3 mL of paste substances or formulations directly onto the CAM, ensuring that at least 50% of the CAM surface area is covered. In cases where the total weight of the test substance at this volume is greater that 0.3 g, 0.3 g of the paste test substance should be used. In either case, the weight of the test substance should be recorded.

6.4 Observations

Observe the reactions on the CAM over a period of 300 seconds. The time for the appearance of each of the noted endpoints should be monitored and recorded, in seconds. Endpoints that should be observed are:

- Hemorrhage (bleeding from the vessels)
- Vascular lysis (blood vessel disintegration)
- Coagulation (intra- and extra-vascular protein denaturation)

Hemorrhage time = observed start (in seconds) of hemorrhage reactions on CAM

Lysis time = observed start (in seconds) of vessel lysis on CAM

Coagulation time = observed start (in seconds) of coagulation formation on CAM

Collection of additional information and data may be useful in further analyses and conducting retrospective studies. To maximize the likelihood of obtaining reproducible results, reference photographs for all endpoints should be available.

7.0 Evaluation of Test Results

The ICCVAM-recommended HET-CAM protocol for prospective studies is the IS(A) analysis method, which is based on development of each of the three HET-CAM endpoints at fixed time intervals of 0.5, 2, and 5 minutes (Luepke 1985).

The numerical time-dependent scores for lysis, hemorrhage, and coagulation (**Table 7-1**) are summed to give a single numerical value indicating the irritation potential of the test substance on a scale with a maximum value of 21.

Table 7-1 Scoring Scheme for Irritation Testing with the HET-CAM Test Method

Effect	Score		
	0.5 min	**2 min**	**5 min**
Lysis	5	3	1
Hemorrhage	7	5	3
Coagulation	9	7	5

For retrospective analyses, data from the HET-CAM test method protocol using the IS(B) analysis method (ICCVAM 2006) could be converted to fixed time points similar to those used for the IS(A) analysis method.

8.0 Criteria for an Acceptable Test

A test is considered acceptable if the negative and positive controls each induce a response that falls within the classification of nonirritating and severely irritating, respectively. Historical control studies indicate that using 0.9% NaCl, as a negative control, the IS value was 0.0. Historical control studies indicate that using 1% SDS and 0.1 N NaOH, as positive controls, the IS values ranged between 10 and 19, respectively.

9.0 Data Interpretation

When using the IS analysis method, the severe irritancy classification for a test substance is assigned when the value is greater than nine.

10.0 Study Report

Information and data that should be included in study reports for the HET-CAM test method include, but are not limited to:

Test and Control Substances

- Chemical name(s) such as the structural name used by the Chemical Abstracts Service (CAS), followed by other names, if known
- The CAS Registry Number (RN), if known
- Purity and composition of the substance or preparation (in percentage(s) by weight)
- Physicochemical properties such as physical state, volatility, pH, stability, chemical class, water solubility relevant to the conduct of the study
- Treatment of the test/control substances prior to testing, if applicable (e.g., warming, grinding)
- Stability, if known

Information Concerning the Sponsor and the Test Facility

- Name and address of the Sponsor
- Name and address of the test facility
- Name and address of the Study Director

Justification of the Test Method and Protocol Used

Test Method Integrity

- The procedure used to ensure the integrity (i.e., accuracy and reliability) of the test method over time (e.g., periodic testing of proficiency substances, use of historical negative and positive control data)

Criteria for an Acceptable Test

- Acceptable concurrent negative control ranges based on historical data
- Acceptable concurrent positive control ranges based on historical data
- If applicable, acceptable concurrent benchmark control ranges based on historical data

Test Conditions

- Experimental starting and completion dates
- Details of test procedure used
- Test concentration(s) used
- Description of any modifications of the test procedure
- Reference to historical data of the model (e.g., negative and positive controls, proficiency substances, benchmark substances)
- Description of evaluation criteria used

Results

- Tabulation of data from individual test samples (e.g., irritancy scores for the test substance and the various controls, including data from replicate repeat experiments as appropriate, and means and ± the standard deviation for each test)

Description of Other Effects Observed

Discussion of the Results

Conclusion

A Quality Assurance Statement for Good Laboratory Practice (GLP)-Compliant Studies

- This statement indicates all inspections made during the study, and the dates any results were reported to the Study Director. This statement also serves to confirm that the final report reflects the raw data.

If GLP-compliant studies are performed, then additional reporting requirements provided in the relevant guidelines (e.g., OECD 1998; EPA 2003b, 2003c; FDA 2003) should be followed.

11.0 References

Balls M, Botham PA, Bruner LH, Spielmann H. 1995. The EC/HO international validation study on alternatives to the Draize eye irritation test. Toxicol In Vitro 9:871–929.

EPA. 2003a. Label Review Manual: 3rd ed. EPA737-B-96-001. Washington, DC:U.S. Environmental Protection Agency.

EPA. 2003b. Good Laboratory Practice Standards. 40 CFR 792.

EPA. 2003c. Good Laboratory Practice Standards. 40 CFR 160.

EU. 2001. Commission Directive 2001/59/EC of 6 August 2001 adapting to technical progress for the 28th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. Official Journal of the European Communities L255:1–333.

FDA. 2003. Good Laboratory Practice for Nonclinical Laboratory Studies. 21 CFR 58.

Gilleron L, Coecke S, Sysmans M, Hansen E, van Oproy S, Marzin D, et al. 1996. Evaluation of a modified HET-CAM assay as a screening test for eye irritancy. Toxicol In Vitro 10:431–446.

Gilleron L, Coecke S, Sysmans M, Hansen E, van Oproy S, Marzin D, et al. 1997. Evaluation of the HET-CAM-TSA method as an alternative to the Draize eye irritation test. Toxicol In Vitro 11:641–644.

ICCVAM. 2006. ICCVAM Test Method Evaluation Report: *In Vitro* Ocular Toxicity Methods for Identifying Severe Irritants and Corrosives. NIH Publication No: 07-4517. Research Triangle Park, NC:National Institute of Environmental Health Sciences.

INVITTOX. 1992. Protocol No. 47: HET-CAM Test. Available: http://ecvam-sis.jrc.it/invittox/published/indexed_47.html.

Luepke N. 1985. Hen's egg chorioallantoic membrane test for irritation potential. Food Chem Toxicol 23:287–291.

OECD. 1998. OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring Number 1: OECD Principles on Good Laboratory Practice [as revised in 1997]. ENV/MC/CHEM(98)17. Paris:Organisation for Economic Co-operation and Development. Available: http://www.oecd.org.

Spielmann H, Liebsch M, Kalweit S, Moldenhauer F, Wirnsberger T, Holzhutter H, et al. 1996. Results of a validation study in Germany on two in vitro alternatives to the Draize eye irritation test, HET-CAM test and the 3T3 NRU cytotoxicity test. ATLA 24:741–858.

UN. 2007. Globally Harmonized System of Classification and Labelling of Chemicals (GHS). New York:United Nations Publications. Available: http://www.unece.org/trans/danger/publi/ghs/ghs-rev02/02files_e.html.

This page intentionally left blank

Appendix B4

ICCVAM-Recommended Protocol for Future Studies Using the Isolated Chicken Eye (ICE) Test Method

This page intentionally left blank

PREFACE

This proposed protocol for measuring corneal damage was developed following a comprehensive test method evaluation process conducted by ICCVAM, which included an international independent scientific peer review of the validation status and scientific validity of the ICE (ICCVAM 2006a,b). It is based primarily on the current protocol used by Menk Prinsen, the original developer of the test method (Prinsen and Koeter 1993; INVITTOX 1994; Balls et al. 1995; Prinsen 1996; Chamberlain et al. 1997). Future studies using the ICE test method could include further characterization of the usefulness or limitations of the ICE in a weight-of-evidence approach for regulatory decision-making. Users should be aware that the proposed test method protocol could be revised based on any additional optimization and/or validation studies that are conducted in the future. ICCVAM recommends that test method users consult the NICEATM-ICCVAM website (http://iccvam.niehs.nih.gov/) to ensure use of the most current test method protocol.

1.0 Purpose and Applicability

The purpose of this protocol is to describe the procedures used to evaluate the potential ocular irritancy of a test substance as measured by its ability to induce toxicity in an enucleated chicken eye. Toxic effects are measured by (1) qualitative assessment of corneal opacity; (2) qualitative measurement of increased retention of fluorescein dye within the eye (permeability); (3) quantitative measurement of increased corneal thickness (swelling); and (4) qualitative evaluation of macroscopic morphological damage to the corneal surface. The opacity, swelling, and permeability assessments following exposure to a test article are assessed individually and then combined to derive an Eye Irritancy Classification.

The focus of this protocol is on the use of the ICE test method for the detection of ocular corrosives and severe irritants, as defined by the U.S. Environmental Protection Agency (EPA; EPA 2003a), European Union (EU; EU 2001), and United Nations Globally Harmonized System (GHS) of Classification and Labelling of Chemicals (UN 2007). Substances other than ocular corrosives and severe irritants (e.g., substances not labeled as irritants and mild/moderate ocular irritants) have been tested using this protocol; however, the ICE test method is not currently considered to be adequately validated for these classes of ocular irritancy as defined by EPA (2003a), EU (2001), and GHS (UN 2007).

2.0 Safety and Operating Precautions

All procedures with chicken eyes should follow the institution's applicable regulations and procedures for handling of human or animal materials, which include, but are not limited to, tissues and tissue fluids. Universal laboratory precautions are recommended, including the use of laboratory coats, eye protection, and gloves. If available, additional precautions required for specific study substances should be identified in the Material Safety Data Sheet for that substance.

3.0 Materials, Equipment, and Supplies

3.1 Source of Chicken Eyes

Spring chickens obtained from a local source (e.g., poultry abattoir), approximately 7 weeks old, male or female, with a weight range of 2.5–3.0 kg (breed not specified).

3.2 Equipment and Supplies

- Custom superfusion apparatus (that will accommodate the eye holders) with a water pump for temperature control
- Dissection equipment (e.g., scissors and forceps)
- Electronic balance
- Eye holders (custom stainless steel clamps)
- Micropipettor and pipette tips
- Mortar and pestle
- Physiological saline
- Slit-lamp microscope with an optical pachymeter equipped with centering lights
- Tissue paper
- Transportation chambers (humidified plastic boxes containing tissues moistened with isotonic saline or water)
- Volumetric flasks
- Peristaltic pump for the saline drip onto the eye

3.3 Solutions

The manufacturer's recommendations with regard to storage temperature and shelf life of stock solutions should be followed. Assay solutions should be prepared volumetrically.

- Fluorescein sodium BP, 2% w/v (also available commercially)
- Isotonic saline (i.e., 0.9% NaCl)
- 4% neutral buffered formaldehyde

4.0 Test Substance Preparation

4.1 Liquid Test Substances

Liquid test substances are typically tested undiluted, but may be diluted if deemed necessary (e.g., as part of the study design). The preferred solvents for diluted substances are either deionized/distilled water or physiological saline. However, alternative solvents may also be used under controlled conditions, but the appropriateness of solvents other than deionized/distilled water or physiological saline must be demonstrated.

4.2 Solid Test Substances

Prior to testing, solid, particulate or granular test substances should be ground as finely as possible in a mortar and pestle.

5.0 Controls

5.1 Negative Controls

A negative control (e.g. deionized/distilled water, isotonic saline, other assay medium) should be included in each experiment in order to detect non-specific changes in the test system, and to ensure that the assay conditions do not inappropriately result in an irritant response.

5.2 Solvent/Vehicle Controls

Solvent/vehicle controls are recommended when solvents/vehicles other than deionized/distilled water, saline, or other assay medium are used to dissolve test substances, in order to demonstrate that the solvent/vehicle is not interfering with the test system.

5.3 Positive Controls

A known ocular irritant is included as a concurrent positive control in each experiment to verify that an appropriate response is induced. As the ICE assay is being used to identify corrosive or severe irritants, the positive control should be a reference substance that induces a severe response in this test method. However, to ensure that variability in the positive control response across time can be assessed, the magnitude of the severe response should not be excessive. Sufficient *in vitro* data for the positive control should be generated such that a statistically defined acceptable range for the positive control can be calculated. If adequate historical ICE test method data are not available for a particular positive control, studies may need to be conducted to provide this information.

Examples of positive controls for liquid test substances are 10% acetic acid or 5% benzalkonium chloride, while examples of positive controls for solid test substances are sodium hydroxide or imidazole.

5.4 Benchmark Controls

Benchmark controls may be useful to demonstrate that the test method is functioning properly for detecting the ocular irritancy potential of chemicals of a specific chemical class or a specific range of responses, or for evaluating the relative irritancy potential of an ocular irritant. Appropriate benchmark controls should have the following properties:

- A consistent and reliable source(s) for the chemical
- Structural and functional similarity to the class of the substance being tested
- Known physical/chemical characteristics
- Supporting data on known effects in animal models
- Known potency in the range of the desired response

6.0 Experimental Design

6.1 Collection and Transport Conditions of Chicken Eyes

Heads of spring chickens should be obtained from a local source (e.g., poultry abattoir). Heads should be removed immediately after sedation of the animals by electric shock and incision of the neck for bleeding. Chicken heads may then be transported to the laboratory at ambient temperature in humidified plastic boxes (i.e., sealed with tissues moistened with isotonic saline) within two hours after they are humanely killed. Once at the laboratory, the eyes may be dissected from each chicken head.

6.2 Preparation of Eyes

a. Carefully remove the eyelids without damaging the cornea. Place a drop of fluorescein sodium BP 2% w/v onto the corneal surface for 10-20 seconds, and then immediately rinse the eye with 20 mL isotonic saline. Examine the fluorescein-treated cornea with a slit-lamp microscope to ensure that the cornea is undamaged (i.e., fluorescein retention and corneal opacity scores < 0.5).

b. If undamaged, further dissect the eye from the eye socket, taking care not to damage the corneal epithelium. When removing the eye from the orbit, a visible portion of the optic nerve should be left attached to the eye.

c. Once removed from the orbit, place the eye on an underpad and cut away the nictitating membrane and other connective tissue.

d. Mount the eyes in stainless steel clamps (one eye per clamp), with the cornea positioned vertically and then transfer each clamp to a chamber in the superfusion apparatus. The chambers of the superfusion apparatus should be temperature controlled at 32 ± 1.5°C with a water pump. Position the clamp in the superfusion apparatus such that the entire cornea is supplied with isotonic saline from a bent stainless steel tube at a rate of 0.10-0.15 mL/minute via a peristaltic pump.

e. After being placed in the superfusion apparatus, the eyes are again examined with a slit-lamp microscope to ensure that they have not been damaged during the dissection procedure. Corneal thickness should also be measured at this time at the corneal apex using the depth measuring device on the slit-lamp microscope. Eyes with (i), a fluorescein retention score of > 0.5; (ii) corneal opacity > 0.5; or, (iii), any additional signs of damage should be replaced. For eyes that are not rejected based on any of these criteria, individual eyes with a corneal thickness deviating more than 10% from the mean value for all eyes are to be rejected. Users should be aware that slit-lamp microscopes could yield different corneal thickness measurements if the slit-width setting is different. The slit-width should be set at 0.095 mm.

f. Once all eyes have been examined and approved, incubate eyes at 32 ± 1.5 °C for 45-60 minutes to equilibrate them to the test system prior to dosing.

6.3 Treatment Groups

Each treatment group and concurrent positive control consists of a minimum of three eyes. The negative control group or the solvent control (if using a solvent other than saline) consists of at least one eye.

6.4 Treatment of Eyes and Observations

6.4.1 Dosing procedure

a. After the equilibration period, record a zero reference measurement for corneal thickness and corneal opacity to serve as a baseline (i.e., time = 0). The fluorescein retention score determined at dissection is used as the baseline measurement.

b. Immediately following the zero reference measurement, apply the test substance to the eye (see Sections 6.4.1.1 and 6.4.1.2).

c. During the dosing procedure, remove the clamp holding the eye from the superfusion apparatus and place it on tissue paper with the cornea facing upwards.

d. Apply the test material for a total of 10 seconds and then rinse the eye with 20 mL isotonic saline at room temperature.

e. After the rinse step, return the eye to the superfusion apparatus.

Liquid test substances

Apply a liquid test substance at 0.03 mL with a micropipettor such that the entire surface of the cornea is covered with the test substance.

Solid test materials

If necessary, grind solid test substances into a fine powder with a mortar and pestle, or comparable grinding tools. Apply 0.03 g of a solid test substance evenly over the entire surface of the cornea.

6.4.2 Endpoint observations

a. Examine the control and test eyes at 30, 75, 120, 180, and 240 minutes (± 5 minutes) after treatment using the criteria and scoring system as indicated in Section 6.4.2.1.

b. Corneal opacity, corneal thickness, and any morphological effects should be evaluated at each time point, while fluorescein retention is determined only at the 30-minute time point.

c. After the final (240 minutes) examination, immerse all eyes in 4% neutral buffered formaldehyde for preservation for possible histopathological examination (if necessary).

d. To maximize the likelihood of obtaining reproducible results, reference photographs for all subjective endpoints (i.e., corneal opacity, fluorescein retention, morphological effects, histopathology) should be readily available.

Criteria and scoring system

The following criteria and scoring system are applied for the assessment of possible effects:

- Corneal swelling is expressed as a percentage and is calculated according to the following formula:

$$\left(\frac{\textit{corneal thickness at time } t \quad - \quad \textit{corneal thickness at time} = 0}{\textit{corneal thickness at time} = 0}\right) \times 100$$

The mean percentage of swelling for all test eyes is calculated for all observation time points. Based on the highest mean score for corneal swelling, as observed at any time point, an overall category score is then given for each test substance.

- Corneal opacity is calculated by using the area of the cornea that is most densely opacified for scoring.

Score	*Observation*
0 =	No opacity
0.5 =	Very faint opacity
1 =	Scattered or diffuse areas; details of the iris are clearly visible
2 =	Easily discernible translucent area; details of the iris are slightly obscured
3 =	Severe corneal opacity; no specific details of the iris are visible; size of the pupil is barely discernible
4 =	Complete corneal opacity; iris invisible

The mean corneal opacity value for all test eyes is calculated for all observation time points.

- Fluorescein retention
  The mean fluorescein retention value for all test eyes is calculated for the 30-minute observation time point only. When test substances have adhered to the cornea, fluorescein retention can be determined whenever the test substance has sufficiently loosened. The following scale is used for scoring:

Score	Observation
0 =	No fluorescein retention
0.5 =	Very minor single cell staining
1 =	Single cell staining scattered throughout the treated area of the cornea
2 =	Focal or confluent dense single cell staining
3 =	Confluent large areas of the cornea retaining fluorescein

- Morphological effects include "pitting" of corneal epithelial cells, "loosening" of epithelium, "roughening" of the corneal surface and "sticking" of the test substance to the cornea. These findings can vary in severity and may occur simultaneously. The classification of these findings is subjective according to the interpretation of the investigator. On the basis of severity of the observed findings, these effects are divided into four categories: 1 = none; 2 = slight; 3 = moderate; 4 = severe.
- A histopathological evaluation of the corneal tissue should be included when the standard ICE endpoints (i.e., corneal opacity, swelling, and fluorescein retention) produce borderline results. A standardized scoring scheme using the formal language of pathology to describe any effects should be included.

7.0 Evaluation of Test Results

Results from the three test method endpoints, corneal opacity, corneal swelling, and fluorescein retention should be evaluated separately (as in Section 9.0), and also combined to generate an Irritancy Classification for a test material (as in Section 10.0).

8.0 Criteria for an Acceptable Test

A test is considered acceptable if the negative and positive controls give an Irritancy Classification that falls within nonirritating and severely irritating, respectively

9.0 Data Interpretation

Interpretation of corneal thickness, corneal opacity, and fluorescein retention using four irritancy categories is done according to the following scales:

9.1 Corneal Thickness

Mean Corneal Swelling (%)	Category
0 to 5	I
> 5 to 12	II
> 12 to 18 (>75 minutes after treatment)	II
> 12 to 18 (≤75 minutes after treatment)	III
> 18 to 26	III
> 26 to 32 (>75 minutes after treatment)	III
> 26 to 32 (≤75 minutes after treatment)	IV
> 32	IV

9.2 Corneal Opacity

Mean Maximum Opacity Score	Category
0.0–0.5	I
0.6–1.5	II
1.6–2.5	III
2.6–4.0	IV

9.3 Fluorescein Retention

Mean Fluorescein Retention Score at 30 minutes post-treatment	Category
0.0–0.5	I
0.6–1.5	II
1.6–2.5	III
2.6–3.0	IV

10.0 Assessment of the Eye Irritancy

The irritancy classification for a test substance is assessed by reading the irritancy classification that corresponds to the combination of categories obtained for corneal swelling, corneal opacity, and fluorescein retention, as presented in the scheme below.

Classification	Combinations of the 3 Endpoints
Severely Irritating	3 x IV
	2 x IV, 1 x III
	2 x IV, I x II*
	2 x IV, I x I*
	Corneal opacity ≥ 3 at 30 min (in at least 2 eyes)
	Corneal opacity = 4 at any time point (in at least 2 eyes)
	Severe loosening of the epithelium (in at least 1 eye)

* Combinations less likely to occur.

11.0 Study Report

The test report should include the following information, if relevant to the conduct of the study:

Test and Control Substances

- Chemical name(s) such as the structural name used by the Chemical Abstracts Service (CAS), followed by other names, if known;
- The CAS Registry Number (RN), if known;
- Purity and composition of the substance or preparation (in percentage[s] by weight), to the extent this information is available;

- Physicochemical properties such as physical state, volatility, pH, stability, chemical class, water solubility relevant to the conduct of the study;
- Treatment of the test/control substances prior to testing, if applicable (e.g., warming, grinding);
- Stability, if known.

Information Concerning the Sponsor and the Test Facility

- Name and address of the sponsor, test facility, and study director;
- Identification of the source of the eyes (i.e., the facility from which they were collected);
- Storage and transport conditions of eyes (e.g., date and time of eye collection, time interval prior to initiating testing);
- If available, specific characteristics of the animals from which the eyes were collected (e.g., age, sex, strain, weight of the donor animal).

Justification of the Test Method and Protocol Used

Test Method Integrity

- The procedure used to ensure the integrity (i.e., accuracy and reliability) of the test method over time (e.g., periodic testing of proficiency substances, use of historical negative and positive control data).

Criteria for an Acceptable Test

- If applicable, acceptable concurrent benchmark control ranges based on historical data.

Test Conditions

- Description of test system used:
- Slit-lamp microscope used (e.g., model);
- Instrument settings for the slit-lamp microscope used:
- Information for the chicken eyes used, including statements regarding their quality;
- Details of test procedure used;
- Test concentration(s) used;
- Description of any modifications of the test procedure;
- Reference to historical data of the model (e.g., negative and positive controls, proficiency substances, benchmark substances);
- Description of evaluation criteria used.

Results

- Description of other effects observed;
- If appropriate, photograph of the eye.

Discussion of the Results

Conclusion

A Quality Assurance Statement for Good Laboratory Practice (GLP)-Compliant Studies

- This statement indicates all inspections made during the study, and the dates any results were reported to the study director. This statement also serves to confirm that the final report reflects the raw data.

If GLP-compliant studies are performed, then additional reporting requirements provided in the relevant guidelines (e.g., OECD 1998; EPA 2003b, 2003c; FDA 2003) should be followed.

12.0 References

Balls M, Botham PA, Bruner LH, Spielmann H. 1995. The EC/HO international validation study on alternatives to the Draize eye irritation test. Toxicol In Vitro 9:871-929.

Chamberlain M, Gad SC, Gautheron P, Prinsen MK. 1997. IRAG Working Group I: Organotypic models for the assessment/prediction of ocular irritation. Food Chem Toxicol 35:23-37.

EPA. 2003a. Label Review Manual: 3rd ed. EPA737-B-96-001. Washington, DC:U.S. Environmental Protection Agency.

EPA. 2003b. Good Laboratory Practice Standards. 40 CFR 792.

EPA. 2003c. Good Laboratory Practice Standards. 40 CFR 160.

EU. 2001. Commission Directive 2001/59/EC of 6 August 2001 adapting to technical progress for the 28th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. Official Journal of the European Communities L255: 1–333.

FDA. 2003. Good Laboratory Practice for Nonclinical Laboratory Studies. 21 CFR 58.

ICCVAM. 2006a. Background Review Document: Current Status of *In Vitro* Test Methods for Identifying Ocular Corrosives and Severe Irritants: Isolated Chicken Eye Test Method. NIH Publication No. 06-4513. Research Triangle Park, NC:National Institute of Environmental Health Sciences, A3-A18. Available: http://iccvam.niehs.nih.gov/docs/ocutox_docs/ocubrd/ice/icebrd.pdf.

ICCVAM. 2006b. ICCVAM Test Method Evaluation Report: *In Vitro* Ocular Toxicity Test Methods for Identifying Severe Irritants and Corrosives. NIH Publication No.: 07-4517. Research Triangle Park, NC:National Institute of Environmental Health Sciences. Available: http://iccvam.niehs.nih.gov/docs/ocutox_docs/OTeval/OTevalrpt.pdf.

INVITTOX. 1994. Protocol No. 80: Chicken enucleated eye test (CEET). Available: http://ecvam-sis.jrc.it/.

OECD. 1998. OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring Number 1: OECD Principles on Good Laboratory Practice [as revised in 1997]. ENV/MC/CHEM(98)17. Paris:Organisation for Economic Co-operation and Development. Available: http://www.oecd.org.

Prinsen MK. 1996. The chicken enucleated eye test (CEET): A practical (pre)screen for the assessment of eye irritation/corrosion potential of test materials. Food Chem Toxicol 34:291-296.

Prinsen MK, Koëter BWM. 1993. Justification of the enucleated eye test with eyes of slaughterhouse animals as an alternative to the Draize eye irritation test with rabbits. Food Chem Toxicol 31:69-76.

UN. 2007. Globally Harmonized System of Classification and Labelling of Chemicals (GHS). 2nd rev. ed. New York:United Publications Publications. Available: http://www.unece.org/trans/danger/publi/ghs/ghs_rev02/02files_e.html.

This page intentionally left blank

Appendix B5

ICCVAM-Recommended Protocol for Future Studies Using the Isolated Rabbit Eye (IRE) Test Method

ICCVAM recommends this IRE test method protocol for nonregulatory, validation, or optimization studies to facilitate collection of consistent data and expand the available database. Exceptions and/or changes to the test method protocol should be accompanied by a scientific rationale.

This page intentionally left blank

PREFACE

The information included in this protocol was derived from protocols used at Unilever Safety and Environmental Assurance Centre, Colworth, United Kingdom (Jones P, personal communication) and at SafePharm Laboratories, Derby, United Kingdom (Whittingham A, personal communication) and from evaluation of IRE protocols reported in the literature (Burton et al. 1981; Price and Andrews 1985; Whittle et al. 1992; INVITTOX 1994; Balls et al. 1995; Chamberlain et al. 1997; Cooper et al. 2001; Jones et al. 2001; Guerriero et al. 2004). Future studies using the IRE test method could include further characterization of the usefulness or limitations of the IRE in a weight-of-evidence approach for regulatory decision-making. Users should be aware that the proposed test method protocol could be revised based on any additional optimization and/or validation studies that are conducted in the future. ICCVAM recommends that test method users consult the NICEATM-ICCVAM website (http://iccvam.niehs.nih.gov/) to ensure use of the most current test method protocol.

1.0 Purpose and Applicability

The purpose of the protocol is to provide details of the essential procedures required to (1) insure induction of corneal irritancy in the enucleated eye of the rabbit by a potentially irritating test substance, (2) evaluate the degree of irritancy, and (3) enable assignment of an appropriate regulatory classification on the potential ocular irritancy of a test substance. Toxic effects in the isolated rabbit eye are measured by (1) subjective assessment of changes in corneal opacity, (2) uptake of fluorescein dye within the cornea (permeability), (3) increased corneal thickness (swelling), and (4) corneal epithelial changes (pitting, sloughing, mottling, etc.) evaluated macroscopically or by slit-lamp. The opacity, swelling, and permeability assessments following exposure to a test substance are assessed individually and are used to determine if the test substance has the potential to induce ocular corrosion or severe irritation.

The focus of this protocol is on the use of the IRE test method for the detection of ocular corrosives and severe irritants, as defined by the U.S. Environmental Protection Agency (EPA 2003a), European Union (EU 2001), and United Nations Globally Harmonized System (GHS) of Classification and Labelling of Chemicals (UN 2007). However, the IRE test method is not currently considered to be adequately validated for classification of ocular irritancy as defined by EPA (2003a), EU (2001), and GHS (UN 2007).

ICCVAM recommends this IRE test method protocol for nonregulatory, validation, or optimization studies to facilitate collection of consistent data and expand the available database. Exceptions and/or changes to the test method protocol should be accompanied by a scientific rationale.

2.0 Safety and Operating Precautions

All procedures with rabbit eyes should follow the institution's applicable regulations and procedures for handling of human or animal substances, which include, but are not limited to, tissues and tissue fluids. Universal laboratory precautions are recommended, including the use of laboratory coats, eye protection, and gloves. If available, additional precautions required for specific study substances should be identified in the Material Safety Data Sheet for that substance.

3.0 Material, Equipment, and Supplies

3.1 Source of Rabbit Eyes

Rabbits should not be bred and sacrificed specifically for use in the IRE test method. Eyes should be obtained from healthy New Zealand White rabbits of either sex weighing 2.5-4.0 kg. To reduce

animal usage, rabbits may be obtained from intra- or extramural laboratories where rabbits may have been used for other purposes (e.g., isolated organ bath, controls) that would not affect ocular tissue, or from a local abattoir where rabbits are typically sacrificed as a food source. Isolated rabbit eyes of exceptional quality without corneal surface defects may be purchased and shipped overnight from a reputable source such as Pel-Freeze Biologicals (Edelhauser H, personal communication). For rapid transfers from laboratory to laboratory within close proximity to each other (1 hour or less), the eyes may be wetted with isotonic saline, or an appropriate buffer (e.g., HBSS without phenol red), secured in position in a hydrated container at room temperature and sealed for shipment. For longer shipments (up to 4 hours), storage at 4°–8°C is recommended. For overnight shipment, storage at 4°–8°C in isotonic saline, or an appropriate buffer (e.g., HBSS without phenol red) with optional antibiotics and an antimycotic is recommended (Vafeas et al. 1998; Chandrasekher et al. 2002).

3.2 Equipment and Supplies

- Chamber, superfusion, Perspex® or similar inert material, water-jacketed temperature-controlled at 32 ± 1.5°C (Burton et al. 1981)
- Drip tubes made from stainless steel tubing (for saline rinsing of cornea)
- Forceps, tissue
- Holders, eye, Perspex or stainless steel with moveable upper jaw
- Magnifying glass
- Plastic tubing, medical or food-grade to supply lines for saline drip tubes
- Pump, peristaltic, 0.1-0.4 mL/minute flow rate adjusted to pump saline in flask in water bath through the saline drip tube
- Pump, peristaltic, approximately 4 L/minute flow rate to pump water through superfusion apparatus and maintain temperature control
- Scissors, fine surgical
- Scissors, surgical enucleation
- Slit-lamp biomicroscope or equivalent
- Optical or ultrasonic pachymeter to quantitatively measure corneal thickness. The optical pachymeter is used in conjunction with the slit-lamp whereas the ultrasonic pachymeter is a stand-alone device.
- Syringe, plastic, 20 ml for eye wash
- Syringe for sodium pentobarbitone administration
- Thermistor (e.g., YSI thermistor, Yellow Spring Co., Inc, OH, USA) to check saline drip temperature
- Tubing, food or medical grade for pumping saline and for connecting to water supply in circulator, sizes may vary with hose fittings
- Water bath, recirculating (capable of maintaining a temperature of 32 ± 1.5°C)
- Weigh Boat, plastic disposable, or a 1 mL disposable plastic syringe with the narrow tip removed

3.3 Solutions

Solutions may be obtained ready prepared from a commercial supplier. Follow the manufacturer's recommendations with regard to storage temperature and shelf life of stock solutions. If necessary, prepare assay solutions volumetrically and store at room temperature unless otherwise noted. Buffers or solutions containing glucose or temperature-sensitive components should be stored at 4°–8°C and equilibrated to room temperature just before use.

- Buffers, physiological salt solution (Hank's, Krebs, etc.)
- Fluorescein, sodium BP (1%–2%), prepared fresh on the day of the experiment
- Physiological (isotonic) saline (0.9%)

- Sodium pentobarbitone
- Sterile deionized/distilled water

4.0 Test Substance Preparation

4.1 Liquid Test Substances

Apply liquid test substances undiluted, although liquid test substances may be diluted if deemed necessary (e.g., as part of the study design). Isotonic saline or standard buffered physiological salt solutions (e.g., Hank's, Krebs, etc.) are the recommended solvents. The appropriateness of solvents other than isotonic saline or standard buffered physiological salt solutions must be demonstrated.

4.2 Solid, Particulate or Granular Test Substances

Grind solid, particulate or granular test substances as fine as possible in a mortar and pestle. The material may be sprinkled on the cornea using a weigh boat or gently compacted in a syringe with the narrow tip removed and then applied. The substance may need to be prewetted and the pH measured (Guest R, personal communication).[2]

5.0 Controls

5.1 Negative Control

A negative control (e.g., distilled water, isotonic saline, other assay medium) is included in each experiment in order to detect non-specific changes in the test system, as well as to provide a baseline for the assay endpoints, and ensure that the assay conditions do not inappropriately result in an irritant response.

5.2 Solvent/Vehicle Controls

Solvent/vehicle controls are recommended when solvents/vehicles other than deionized/distilled water, saline, or other assay medium are used to dissolve test substances, in order to demonstrate that the solvent/vehicle is not interfering with the test system.

5.3 Positive Controls

A known ocular irritant is included in each experiment to verify that an appropriate response is induced. If the IRE assay is being used only to identify corrosive or severe irritants, then the positive control should be a reference substance that induces a severe response *in vivo* as well as in the IRE. However, to ensure that variability in the positive control response across time can be assessed, the magnitude of the severe response should not be excessive. The selection of positive control test substances should be based on the availability of high quality *in vivo* data. For test substances being tested in liquid or solid form, a corresponding liquid or solid positive control should be included in the test.

5.4 Benchmark Controls

Benchmark controls may be useful to demonstrate that the test method is functioning properly for detecting the ocular irritancy potential of substances of a specific chemical class or a specific range of responses, or for evaluating the relative irritancy potential of an ocular irritant. Appropriate

---

[2] Since the isolated eye has less moisture content than the eye *in situ* and compounds that dissociate or hydrolyze could produce false negatives due to reduced dissociation or hydrolysis in the isolated eye.

benchmark controls should be chosen based on high quality *in vivo* test results and have the following properties:

- A consistent and reliable source(s)
- Structural and functional similarity to the class of substance being tested
- Known physical/chemical characteristics
- Supporting data on known effects in the *in vivo* rabbit eye test
- Known potency in the range of the desired response

6.0 Experimental Design

6.1 Treatment Groups

Use at least three eyes for each test substance and three eyes for each of the controls in the study. The controls must be tested concurrently with the test substance.

6.2 Eye Selection and Preparation

a. For each assay, use a number of animals adequate to provide at least three eyes for each test substance and three eyes for each of the various controls considering rejection levels of suitable eyes to be as high as 25% in some cases. All isolated eyes should be randomly distributed within experimental groups, particularly when both eyes from the same rabbit are used.

b. Examine the rabbit corneas *in vivo* macroscopically and microscopically and, if the eyes are accepted to be free of imperfections, measure the initial corneal thickness (Reading T-2; *in vivo* reading, if possible). In some cases, rabbits may be euthanized commercially and this *in vivo* reading may not be possible. In those cases, a pre-equilibration reading (T-1) is sufficient (**Section 6.3**).

c. Euthanize the rabbits humanely by injection of a lethal dose of sodium pentobarbitone into the marginal ear vein. Follow the institution's applicable regulations and procedures regarding euthanasia. A typical lethal dose for rabbits is 200 mg/kg, administered intravenously. Remove each eye by dissection of the conjunctiva and the optic nerve (leave approximately a 5–10 mm section of the nerve to prevent loss of intraocular pressure) after deflection of the nictitating membrane.

d. Rinse the orbit occasionally with saline during the dissection to prevent drying and afterwards to remove any adherent tissue.

e. Ship eyes obtained from external sources in saline or an appropriate buffer (e.g., HBSS without phenol red) at an appropriate temperature (4 -8°C for shipment over periods greater than 1 hour or 25 ± 5°C for shipment over a period of 1 hour or less) in a humidified, sealed container to prevent drying of the corneas. For longer shipments periods (e.g., overnight), antibiotics with an antimycotic may be needed (Vafeas et al. 1998; Chandrasekher et al. 2002).

f. The method of euthanasia and any prior pharmacological or physiological treatment of the animals for eyes shipped from external sources are noted and the eyes are inspected microscopically and macroscopically for imperfections.

g. If there is any doubt that the cornea is free of imperfections, apply a 1%–2% solution of sodium fluorescein BP followed immediately by a gentle, but thorough rinse with physiological saline (a time insufficient for actual penetration of fluorescein) to identify corneal imperfections.

h. Once they have been inspected and are deemed to be free of corneal defects, the eyes are clamped into the holders (one eye per holder) with the cornea in a vertical position, without altering the *in vivo* orientation of the eyeball, and placed in the maintenance chamber (see **Figure 6-1** and **Figure 6-2**).

i. The eyes are equilibrated for 30 to 45 minutes at 32 ± 1.5°C.

Figure 6-1 Isolated Rabbit Eye Equilibration Apparatus

Photo provided courtesy of R. Guest

Figure 6-2 Isolated Rabbit Eye Holder

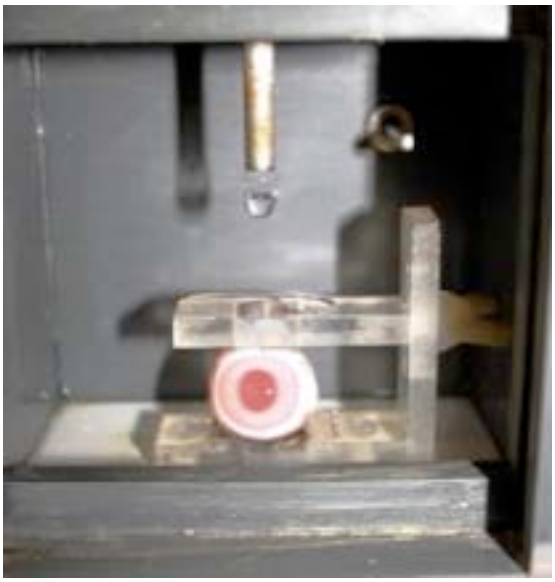


Photo provided courtesy of R. Guest

6.3 Pretreatment Measurements

a. Measure the corneal thickness (Reading T-1) before equilibration. Any eyes in which corneal swelling has exceeded 7% relative to *in vivo* values are discarded and replaced (Reading T-2; **Section 6.2**).

b. The corneal thickness is measured again after equilibration and just prior to application of the test substance. This will become Reading T0 (**Section 6.3**). If a significant amount of time (3 ± 1 hours) has elapsed between post-equilibration and application, any eyes that have swelling >7% relative to the post-equilibration value (T0) should be replaced. If an ultrasonic pachymeter is used which requires direct contact with the cornea, an initial measurement and a post-equilibration reading may be necessary to minimize the possibility of damage to the cornea (Guest R, personal communication).

6.4 Application of Test Substances

a. Remove the holder from the cell where the eye is held in a vertical position, then reposition the eye with the cornea in the horizontal plane (i.e., facing upward) and apply the test substance (premoistened, if necessary) directly on the corneal surface immediately.

b. For liquid substances, apply 0.1 mL of undiluted test substance using a syringe over as much of the entire corneal surface as possible.

c. For solid substances, sprinkle a volume of 0.1 mL (not exceeding 100 mg) of neat test substance pulverized to a fine powder or dust over the entire cornea using a plastic weigh boat or other means of delivery (e.g., from a 1 mL disposable syringe with the tip removed). Record the mean weight of material that is applied to each eye.

d. Adjust the concentration, volume or weight if necessary for compounds with known physical characteristics that may interfere with the test (e.g., viscous substances or solids that irreversibly adhere to the cornea and cannot be washed off).

e. Apply 0.1 mL of physiological saline (prewarmed to 32°C) to the control eye.

f. For liquid positive control substances, apply 0.1 mL using a syringe over as much of the entire corneal surface as possible.

g. For solid positive control substances, sprinkle 0.1 g pulverized to a fine powder or dust over the entire cornea using a plastic weigh boat or other means of delivery.

h. Allow the test substance, the positive control, and the negative control to remain in contact with the cornea for 10 ± 2 seconds.

i. Rinse each eye with 20 ml of physiological saline (prewarmed to 32°C) using a syringe and place the eye holder back in the cell of the maintenance chamber.

j. Return the saline drip tube to its original position to bathe the cornea between measurement periods.

k. Repeat these procedures for subsequent treated and control eyes.

6.5 Endpoint Observations

6.5.1 Corneal opacity and area

a. With the aid of the light source from the slit-lamp (diffuse illumination), examine each eye macroscopically at each time point (0.5, 1, 2, 3, 4 hours), assess the extent of corneal injury, noting signs of sloughing, mottling, pitting or other signs of epithelial damage. Identify focal areas for slit-lamp evaluation.

b. Examine each eye microscopically at each time point (0.5, 1, 2, 3 and 4 hours) using a slit-lamp set with a narrow slit width and score corneal opacity and area involvement according to the scoring system found in **Table 6-1**.

6.5.2 Corneal swelling

a. Measure corneal thickness using the depth measuring attachment or ultrasonic pachymeter before treatment (as described previously) and at each time point post-treatment.

b. Calculate corneal swelling based on the percent change in corneal thickness over time according to the following formula:
[(Corneal Thickness at Time T/Corneal Thickness at Time T0)-1] x 100%

6.5.3 Corneal epithelial observations

a. Examine the cornea macroscopically or by slit-lamp microscopically at each time point for sloughing, mottling, pitting or other signs of epithelial damage.

b. To maximize the likelihood of obtaining reproducible results, reference photographs for all subjective endpoints (i.e., corneal opacity, fluorescein retention, morphological effects, histopathology) should be readily available.

Table 6-1 Evaluation of Corneal Irritation[1]

Description	
Cornea	**Individual Score**
Normal cornea. Appears with the slit-lamp adjusted to a narrow slit image as having a bright gray line on the epithelial surface and a bright gray line on the endothelial surface with a marble-like gray appearance of the stroma.	0
Some loss of transparency. Only the anterior half of the stroma is involved as observed with an optical section of the slit-lamp. The underlying structures are clearly visible with diffuse illumination, although some cloudiness can be readily apparent with diffuse illumination.	1
Moderate loss of transparency. In addition to involving the anterior stroma, the cloudiness extends all the way to the endothelium. The stroma has lost its marble-like appearance and is homogenously white. With diffuse illumination, underlying structures are clearly visible.	2
Involvement of the entire thickness of the stroma with endothelium intact. With optical section, the endothelial surface is still visible. However, with diffuse illumination the underlying structures are just barely visible (to the extent that the observer is still able to grade flare and iritis, observe for pupillary response, and note lenticular changes).	3
Involvement of the entire thickness of the stroma with endothelium damaged. With the optical section, cannot clearly visualize the endothelium. With diffuse illumination, the underlying structures cannot be seen. Cloudiness removes the capability for judging and grading flare, iritis, lenticular changes, and pupillary response.	4
Corneal area	**Individual Score**
Normal cornea with no area of cloudiness	0
1% to 25% area of stromal cloudiness	1
26% to 50% area of stromal cloudiness	2
51% to 75% area of stromal cloudiness	3
76% to 100% area of stromal cloudiness	4
Overall Corneal Opacity/Area	**Product Score**
Corneal Opacity x Area[2]	Maximum of 16

[1] From: Hackett and McDonald (1991).

[2] The overall corneal opacity score is the product of the corneal opacity score and the corneal area score. The product of individual scores of 1 and 4 (Product Score of 4) or 2 and 2 (Product Score of 4), for example, would each qualify for a severe irritant rating based on the overall corneal opacity/area score.

c. Additional endpoints such as histopathology to look at each of the various corneal tissue layers (i.e., epithelium, Bowman's layer, stroma, Descemet's layer, and endothelium) or confocal microscopy with live/dead cell staining may be used to corroborate or to re-evaluate the actual depth of injury, particularly where equivocal results may have been obtained by use of existing endpoints or where the irritancy of a substance falls into the interface between a severe and nonsevere irritant. A standardized scoring scheme using the formal language of pathology to describe any effects should be included.

6.5.4 Fluorescein penetration

- At the end of the 4-hour testing period or earlier score each cornea for fluorescein penetration using a 10 ± 2.0 seconds application followed by a thorough rinse with physiological saline or negative control buffer (**Table 6-2**).

Table 6-2 Fluorescein Penetration Scoring System[1]

Description	**Individual Scores (Area/Intensity)**
Negligible — No staining.	0
Slight staining confined to small focal area. Some loss of detail in underlying structures with diffuse illumination.	1
Moderate staining confined to a small focal area. Some loss of detail in underlying structures on diffuse illumination.	2
Marked staining involving a larger portion of the cornea. Underlying structures are barely visible but not completely obliterated with diffuse illumination.	3
Extreme staining with no visibility of underlying structures.	4
Fluorescein Penetration	**Product Score**
Fluorescein Area x Intensity	**Maximum of 16**

[1] From: Hackett and McDonald (1991).

7.0 Evaluation of Test Results

Using the scores obtained from the endpoints evaluated (as described above), determine if the test substance meets the criteria for a corrosive or severe ocular irritant using the decision criteria provided in **Table 8-1**.

8.0 Criteria for an Acceptable Test

- If, in the course of evaluation of three eyes, there is significant disagreement in the results between eyes, repeat the experiment and calculate the mean for all six determinations to assess overall damage.
- Changes in control eyes greater than 7% during the 4-hour observation period warrant rejection of the experiment.
- A test is considered acceptable if the negative control produces either no effect or only slight or marginal effects on the various parameters and the positive control produces a severe irritant effect as defined in **Table 8-1**.

Table 8-1 Decision Criteria for Determination of Severe Irritants: Overall Scoring System for Corneal Damage and Irritation[1]

Ocular Parameter	Cut-off Value to Detect Severe Eye Irritants
Maximum Corneal Opacity[2] Cloudiness x Area	Greater than or equal to a score of 3
Maximum Fluorescein Uptake[3] Intensity x Area	Greater than or equal to a score of 4
Mean Corneal Swelling[4] 0.5 hours 1 hour 2 hours 3 hours 4 hours	Greater than or equal to 25%
Corneal Epithelial Observations[5]	Any pitting, mottling, or sloughing

[1] From: Guerriero et al., 2002
[2] Represents maximum score obtained in 3 eyes
[3] Represents maximum score obtained in 3 eyes
[4] Represents mean swelling calculated for 3 eyes
[5] Represents information obtained for any single animal

- Control charts should be used to monitor historical responses and calculate acceptable ranges for negative and positive controls, and benchmark controls when used, over time and across laboratories. These ranges should be updated frequently to adjust test acceptance criteria for individual control substances. An acceptable test would then have positive or benchmark controls that fell within these acceptable ranges.

9.0 Data Interpretation

Test substances meeting or exceeding the criteria for severe irritation defined in **Table 8-1** in an acceptable test (as defined in **Section 8.0**) are identified as severe irritants. Test substances not meeting these cut-off criteria in an acceptable test are identified as nonsevere irritants. Benchmark substances are recommended for comparing the responses of test substances of different product or chemical classes. It may be useful to carefully evaluate the pattern of responses in the four endpoints.

10.0 Study Report

The test report should include the following information, if relevant to the conduct of the study:

Test and Control Substances

- Chemical name(s) such as the structural name used by the Chemical Abstracts Service (CAS), followed by other names, if known
- The CAS Registry Number (RN), if known
- Purity and composition of the substance or preparation (in percentage(s) by weight)
- Physicochemical properties such as physical state, volatility, pH, stability, chemical class, water solubility relevant to the conduct of the study
- Treatment of the test/control substances prior to testing, if applicable (e.g., warming, grinding)
- Stability, if known

Information Concerning the Sponsor and the Test Facility

- Name and address of the sponsor
- Name and address of the facility
- Name and address of the Study Director

Justification of the Test Method and Protocol Used

Test Method Integrity

- The procedure used to ensure the integrity (i.e., accuracy and reliability) of the test method over time (e.g., periodic testing of proficiency substances, use of historical negative and positive control data)

Criteria for an Acceptable Test

- Acceptable concurrent negative control ranges based on historical data
- Acceptable concurrent positive control ranges based on historical data
- If applicable, acceptable concurrent benchmark control ranges based on historical data

Test Conditions

- Description of test system used
- Complete supporting information for the enucleated rabbit eyes used including statements regarding their quality
- Details of test procedure used
- Test concentration(s) used
- Description of any modifications of the test procedure
- Reference to historical data of the model (e.g., negative and positive controls, proficiency substances, benchmark substances)
- Description of evaluation criteria used

Results

- Tabulation of data from individual test samples (e.g., irritancy scores for the test substance and the various controls, including data from replicate repeat experiments as appropriate, and means and ± the standard deviation for each trial)

Description of Other Effects Observed

Discussion of the Results

Conclusion

A Quality Assurance Statement for Good Laboratory Practice (GLP)-Compliant Studies

- This statement indicates all inspections made during the study, and the dates any results were reported to the Study Director. This statement also serves to confirm that the final report reflects the raw data.

If GLP-compliant studies are performed, then additional reporting requirements provided in the relevant guidelines (e.g., OECD 1998; EPA 2003b, 2003c; FDA 2003) should be followed.

11.0 References

Balls M, Botham PA, Bruner LH, Speilmann H. 1995. The EC/HO international validation study on alternatives to the Draize eye irritation test. Toxicol In Vitro. 9871-929.

Burton ABG, York M, Lawrence, RS. 1981. The *in vitro* assessment of severe eye irritants. Food Cosmetic Toxicol 19:471-480.

Chamberlain M., Gad SC, Gautheron P, Prinsen MK. 1997. Organotypic models for the assessment/prediction of ocular irritation. Food Chem Toxicol. 35:23-37.

Chandrasekhar G, Ma X, Lallier TE, Bazan HEP. 2002. Delay of corneal epithelial wound healing and induction of keratocyte apoptosis by platelet-activating factor. Invest Ophth Vis Sci. 43:1422–1428.

Cooper KJ, Earl LK, Harbell J, Raabe H. 2001. Prediction of the ocular irritancy of prototype shampoo formulations by the isolated rabbit eye (IRE) test and bovine corneal opacity and permeability assay (BCOP) assay. Toxicol In Vitro. 15:95-103.

EPA. 2003a. Label Review Manual: 3rd ed. EPA737-B-96-001. Washington, DC:U.S. Environmental Protection Agency.

EPA. 2003b. Good Laboratory Practice Standards. 40 CFR 792.

EPA. 2003c. Good Laboratory Practice Standards. 40 CFR 160.

EU. 2001. Commission Directive 2001/59/EC of 6 August 2001 adapting to technical progress for the 28th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. Official Journal of the European Communities L255:1–333.

FDA. 2003. Good Laboratory Practice for Nonclinical Laboratory Studies. 21 CFR 58.

Guerriero FJ, Seaman CW, Olsen MJ, Guest R, Whittingham A. 2004. Retrospective assessment of the rabbit enucleated eye test (REET) as a screen to refine worker safety studies [Abstract No. 1282]. Toxicol Sci (The Toxicologist Supplement). 78(1-S).

Hackett RB, McDonald TO. 1991. Eye irritation. In: Advances in Modern Toxicology: Dermatoxicology. 4th ed. (Marzulli F, Maibach H, eds.). Washington, DC:Hemisphere Publishing Corporation, 749–815.

INVITTOX. 1994. Protocol No. 85: Rabbit Enucleated Eye Test. ECVAM. Available: http://ecvam-sis.jrc.it/.

Jones PA, Budynsky E, Cooper KJ, Decker D, Griffiths HA, Fentem JH. 2001. Comparative evaluation of five *in vitro* tests for assessing the eye irritation potential of hair-care products. ATLA 29:669–692.

OECD. 1998. OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring Number 1: OECD Principles on Good Laboratory Practice. (as revised in 1997). ENV/MC/CHEM(98)17. Paris: Organisation for Economic Co-operation and Development. Available: http://www.oecd.org.

Price JB, Andrews IJ. 1985. The *in vitro* assessment of eye irritancy using isolated eyes. Food Chem Toxicol 25:313–315.

UN. 2007. Globally Harmonized System of Classification and Labelling of Chemicals (GHS). New York:United Nations Publications. Available: http://www.unece.org/trans/danger/publi/ghs/ghs_rev02/02files_e.html.

Vafeas C, Mieyal PA, Urbano F, Falck JR, Chauhan K, Berman M, et al. 1998. Hypoxia stimulates the synthesis of cytochrome P450-derived inflammatory eicosanoids in rabbit corneal epithelium. J Pharmacol Exp Ther. 287:903–910.

Whittle E, Basketter D, York M, Kelly L, Hall T, McCall J, et al. 1992. Findings of an interlaboratory trial of the enucleated eye method as an alternative to the eye irritation test. Toxicol Methods 2:30–41.

Appendix C

Background Review Document
Current Status of *In Vitro* Test Methods
for Identifying Mild/Moderate Ocular Irritants:
Bovine Corneal Opacity and Permeability Test Method

This page intentionally left blank

Background Review Document
Current Status of *In Vitro* Test Methods for Identifying Mild/Moderate Ocular Irritants: Bovine Corneal Opacity and Permeability Test Method

Interagency Coordinating Committee on the Validation of Alternative Methods

National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods

National Institute of Environmental Health Sciences
National Institutes of Health
U.S. Public Health Service
Department of Health and Human Services

2010

National Toxicology Program
P.O. Box 12233
Research Triangle Park, NC 27709

This page intentionally left blank

Table of Contents

List of Tables **C-9**

List of Figures **C-13**

List of Acronyms and Abbreviations **C-14**

Interagency Coordinating Committee on the Validation of Alternative Methods: Agency Representatives **C-16**

Acknowledgements **C-17**

Preface **C-21**

Executive Summary **C-23**

1.0 Introduction **C-29**

1.1 Background C-29

1.2 Use of the BCOP Test Method in Overall Strategy of Hazard or Safety Assessment C-30

1.3 Validation of the BCOP Test Method C-30

1.4 Search Strategies and Selection of Citations for the BCOP BRD C-30

2.0 Bovine Corneal Opacity and Permeability Test Method Protocol Components **C-32**

2.1 Overview of How the BCOP Test Method is Conducted C-32

3.0 Substances Used for Validation of the Bovine Corneal Opacity and Permeability Test Method **C-34**

4.0 *In Vivo* Reference Data Used for an Assessment of Test Method Accuracy **C-37**

4.1 *In Vivo* Classification Criteria Used for BRD Analysis C-38

4.2 *In Vivo* Data Quality C-40

5.0 Bovine Corneal Opacity and Permeability Test Method Data and Results **C-41**

5.1 Availability of Copies of Original Data Used to Evaluate the Accuracy and Reliability C-41

5.2 Description of the Statistical Approaches Used to Evaluate the Resulting Data C-41

5.3 Summary of Results C-42

5.4 Use of Coded Chemicals and Compliance with GLP Guidelines.....C-42

6.0 Bovine Corneal Opacity and Permeability Test Method Accuracy C-43

6.1 Ability to Distinguish Ocular Corrosives and Severe Irritants from All Other Classes C-43

6.2 GHS Classification System: BCOP Test Method Accuracy.....C-44

6.2.1 Identification of Category 1 Substances (Ocular Corrosives/Severe Irritants).....C-44

6.2.2 Identification of Category 2A Substances (Moderate Ocular Irritants) C-44

6.2.3 Identification of Category 2B Substances (Mild Ocular Irritants) C-45

6.2.4 Identification of Substances Not Classified as Irritant.....C-45

6.2.5 Overall Correct Classification C-45

6.2.6 Ability to Distinguish Substances Not Classified as Irritant from All Other Classes C-45

6.2.7 Discordant Results According to the GHS Classification System C-48

6.3 EPA Classification System: BCOP Test Method Accuracy C-54

6.3.1 Identification of Category I Substances (Ocular Corrosives/Severe Irritants).....C-54

6.3.2 Identification of Category II Substances (Moderate Ocular Irritants) C-54

6.3.3 Identification of Category III Substances (Mild Ocular Irritants) C-54

6.3.4 Identification of Category IV Substances C-54

6.3.5 Ability to Distinguish Category IV from All Other Classes C-54

6.3.6 Discordant Results According to the EPA Classification System C-59

6.4 EU Classification System: BCOP Test Method Accuracy C-62

6.4.1 Identification of R41 Substances (Ocular Corrosives/Severe Irritants).....C-62

6.4.2 Identification of R36 Substances (Irritants) C-62

6.4.3 Identification of Not Labeled Substances C-64

6.4.4 Ability to Distinguish Not Labeled Substances from All Other Classes.....C-64

6.4.5 Discordant Results According to the EU Classification System C-67

6.5 FHSA Classification System: BCOP Test Method Accuracy C-72

6.5.1 Ability to Distinguish Not Labeled Substances from All Other Classes C-72

6.5.2 Discordant Results According to the FHSA Classification System C-74

7.0 Bovine Corneal Opacity and Permeability Test Method Reliability.... C-78

7.1 Interlaboratory Reproducibility of Hazard Classification Category Using the GHS Classification System C-78

7.2 Interlaboratory Reproducibility of Hazard Classification Category Using the EPA Classification System C-84

7.3 Interlaboratory Reproducibility of Hazard Classification Category Using the EU Classification System....C-89

8.0 Bovine Corneal Opacity and Permeability Test Method Data Quality C-93

8.1 Adherence to National and International GLP Guidelines C-93

9.0 Reports in the Peer-Reviewed Literature C-94

10.0 Animal Welfare Considerations (Reduction, Refinement, and Replacement) C-95

10.1 How the BCOP Test Method Will Reduce, Refine, or Replace Animal Use C-95

10.2 Requirement for the Use of Animals C-95

11.0 Practical Considerations C-96

12.0 References C-97

13.0 Glossary C-100

Annex I Characterization of Substances Tested in the BCOP Test Method ... C-109

I-1 Chemical and Product Classes of Substances Tested in the BCOP Assay C-111

I-2 Components of Formulations Tested in Gettings et al. (1996)....C-125

I-3 Components of Formulations Tested in Swanson et al. (1995)....C-137

I-4 Components of Formulations Tested in Swanson and Harbell (2000) C-143

I-5 Components of AMCP Formulations Tested C-147

Annex II *In Vitro* Data for Substances Tested in the BCOP Test Method C-161

II-1 BCOP Data Sorted by Reference C-163

II-2 BCOP Data Sorted by Substance Name C-215

Annex III Comparison of *In Vivo* and *In Vitro* Ocular Irritancy Classifications C-269

III-1 BCOP Data Sorted by Reference C-271

III-2 BCOP Data Sorted by Substance Name C-285

List of Tables

Table 1 Evaluation of the Performance of the BCOP Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by GHS, EPA, and EU Classification Systems C-26

Table 2 Accuracy of the BCOP Test Method in Distinguishing Substances Not Labeled as Irritants from All Other Irritant Classes, as Defined by the GHS, EPA, EU, and FHSA Classification Systems C-27

Table 2-1 *In Vitro* Ocular Irritancy Classification Scheme for the BCOP Test Method (ICCVAM 2006b) C-32

Table 2-2 *In Vitro* Ocular Irritancy Classification Scheme for the BCOP Test Method (AMCP BRD 2008 Submission) C-33

Table 3-1 Chemical Classes Tested in the BCOP Test Method C-35

Table 3-2 Product Classes Tested in the BCOP Test Method..... C-36

Table 4-1 FHSA Classification System (16 CFR 1500.42) C-39

Table 4-2 Proposed FHSA "Proportionality" Criteria C-40

Table 6-1 Accuracy of the BCOP Test Method in Distinguishing Ocular Corrosives/Severe Irritants from All Other Categories, as Defined by the EPA, GHS, and EU Classification Systems..... C-44

Table 6-2 Performance of the BCOP Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by EPA, GHS, and EU Classification Systems..... C-46

Table 6-3 Performance of the BCOP Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the GHS Classification System, by Study and Overall..... C-47

Table 6-4 Accuracy of the BCOP Test Method in Distinguishing Substances Not Labeled as Irritants from All Other Irritant Classes, as Defined by the GHS Classification System, by Study and Overall..... C-48

Table 6-5 Under- and Overprediction of the BCOP Test Method Using the GHS Classification System in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method by Chemical Class or Physical Property..... C-50

Table 6-6 Performance of the BCOP Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined

by the GHS Classification System, with Discordant Chemical and Physical Classes Excluded C-52

Table 6-7 Accuracy of the BCOP Test Method in Distinguishing Substances Not Labeled as Irritants from All Other Irritant Classes, as Defined by the GHS Classification System, with Discordant Chemical and Physical Classes Excluded C-53

Table 6-8 Performance of the BCOP Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the EPA Classification System, by Study and Overall C-56

Table 6-9 Accuracy of the BCOP Test Method in Distinguishing Category IV Ocular Irritants from All Other Irritant Classes, as Defined by the EPA Classification System, by Study and Overall C-57

Table 6-10 BCOP False Negative Substances C-58

Table 6-11 Under- and Overprediction of the BCOP Test Method Using the EPA Classification System in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method by Chemical Class or Physical Property C-60

Table 6-12 Performance of the BCOP Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the EPA Classification System, with Discordant Chemical and Physical Classes Excluded C-63

Table 6-13 Accuracy of the BCOP Test Method in Distinguishing Category IV Ocular Irritants from All Other Irritant Classes, as Defined by the EPA Classification System, with Discordant Chemical and Physical Classes Excluded C-64

Table 6-14 Performance of the BCOP Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the EU Classification System, by Study and Overall C-65

Table 6-15 Accuracy of the BCOP Test Method in Distinguishing Not Labeled Substances from All Other Irritant Classes, as Defined by the EU Classification System, by Study and Overall C-66

Table 6-16 Under- and Overprediction of the BCOP Test Method Using the EU Classification System in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method by Chemical Class or Physical Property C-68

Table 6-17 Performance of the BCOP Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the EU Classification System, with Discordant Chemical and Physical Classes Excluded C-70

Table 6-18 Accuracy of the BCOP Test Method in Distinguishing Substances Not Labeled as Irritants from All Other Irritant Classes, as Defined by the EU Classification System, with Discordant Chemical and Physical Classes Excluded C-71

Table 6-19 Accuracy of the BCOP Test Method in Distinguishing Substances Not Labeled as Irritants from All Other Irritant Classes, as Defined by the FHSA-20% Classification System, by Study and Overall C-73

Table 6-20 Accuracy of the BCOP Test Method in Distinguishing Not Labeled Substances from All Other Irritant Classes, as Defined by the FHSA-67% Classification System, by Study and Overall C-75

Table 6-21 Accuracy of the BCOP Test Method in Distinguishing Substances Not Labeled as Irritants from All Other Irritant Classes, as Defined by the FHSA-20% Classification System, with Discordant Chemical and Physical Classes Excluded C-76

Table 6-22 Accuracy of the BCOP Test Method in Distinguishing Substances Not Labeled as Irritants from All Other Irritant Classes, as Defined by the FHSA-67% Classification System, with Discordant Chemical and Physical Classes Excluded C-77

Table 7-1 Reliability of the BCOP Test Method in Predicting Not Labeled Ocular Substances or Corrosives/Severe/Moderate/Mild Irritants, as Defined by the GHS Classification System, by Study C-80

Table 7-2 Interlaboratory Variability of the BCOP Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the GHS Classification System, by Study C-82

Table 7-3 Reliability of the BCOP Test Method in Predicting Not Labeled Ocular Substances or Corrosives/Severe/Moderate/Mild Irritants, as Defined by the EPA Classification System, by Study C-85

Table 7-4 Interlaboratory Variability of the BCOP Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the EPA Classification System, by Study C-87

Table 7-5 Reliability of the BCOP Test Method in Predicting Not Labeled Ocular Substances or Corrosives/Severe/Moderate Irritants, as Defined by the EU Classification System, by Study C-90

Table 7-6 Interlaboratory Variability of the BCOP Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the EU Classification System, by Study..... C-92

List of Figures

Figure 1-1 GHS Testing Strategy for Serious Eye Damage and Eye Irritation..... C-31

List of Acronyms and Abbreviations

AMCP	Antimicrobial cleaning product
BCOP	Bovine corneal opacity and permeability
BRD	Background review document
CASRN	Chemical Abstracts Service Registry Number
CPSC	(U.S.) Consumer Product Safety Commission
CV	Coefficient of variation
EC	European Commission
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
EC/HO	European Commission/British Home Office
ECVAM	European Centre for the Validation of Alternative Methods
EEC	European Economic Community
EPA	(U.S.) Environmental Protection Agency
EU	European Union
FDA	(U.S.) Food and Drug Administration
FHSA	U.S. Federal Hazardous Substances Act
FR	*Federal Register*
g	Gram
GHS	Globally Harmonized System of Classification and Labelling of Chemicals
GLP	Good Laboratory Practice
HET-CAM	Hen's egg test – chorioallantoic membrane
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ICE	Isolated chicken eye
IRE	Isolated rabbit eye
IVIS	*In vitro* irritancy score
JaCVAM	Japanese Center for the Validation of Alternative Methods
μg	Microgram
μL	Microliter

μm	Micrometer
MAS	Maximum average score
MeSH	(National Library of Medicine) Medical Subject Headings
mL	Milliliter
MMAS	Modified maximum average score
NA	Not applicable
NC	Not Classified (as irritant)
NICEATM	National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
NIEHS	National Institute of Environmental Health Sciences
NL	Not Labeled (as irritant)
NTP	(U.S.) National Toxicology Program
OD	Optical density
OECD	Organisation for Economic Co-operation and Development
OPPTS	Office of Prevention, Pesticides and Toxic Substances
OSHA	Occupational Safety and Health Administration
OTWG	Ocular Toxicity Working Group
r	rho (correlation coefficient)
SCNM	Study criteria not met
SD	Standard deviation
TG	Test Guideline
UN	United Nations

Interagency Coordinating Committee on the Validation of Alternative Methods: Agency Representatives

Agency for Toxic Substances and Disease Registry
* Moiz Mumtaz, Ph.D.
Bruce Fowler, Ph.D.
Edward Murray, Ph.D.
Eric Sampson, Ph.D.

Consumer Product Safety Commission
* Marilyn L. Wind, Ph.D. (Chair)
+ Kristina Hatlelid, Ph.D.
Joanna Matheson, Ph.D.

Department of Agriculture
* Jodie Kulpa-Eddy, D.V.M. (Vice-Chair)
+ Elizabeth Goldentyer, D.V.M.

Department of Defense
* Robert E. Foster, Ph.D.
+ Patty Decot
Harry Salem, Ph.D.
Peter J. Schultheiss, D.V.M., DACLAM

Department of Energy
* Michael Kuperberg, Ph.D.
+ Marvin Stodolsky, Ph.D.

Department of the Interior
* Barnett A. Rattner, Ph.D.
+ Sarah Gerould, Ph.D. (to Feb. 2009)

Department of Transportation
* George Cushmac, Ph.D.
+ Steve Hwang, Ph.D.

Environmental Protection Agency
Office of Pesticide Programs
* John R. "Jack" Fowle III, Ph.D., DABT
+ Vicki Dellarco, Ph.D.
+ Tina Levine, Ph.D.
Deborah McCall
Christine Augustyniak, Ph.D. (*U.S. Coordinator, OECD Test Guidelines Program*)
Office of Pollution Prevention and Toxics
Jerry Smrchek, Ph.D. (*U.S. Coordinator, OECD Test Guidelines Program*, to July 2009)
Office of Research and Development
Suzanne McMaster, Ph.D. (to Dec. 2008)
Julian Preston, Ph.D. (to July 2009)
Stephanie Padilla, Ph.D. (to July 2009)
Office of Science Coordination and Policy
Karen Hamernik, Ph.D. (to July 2009)

* Principal agency representative
+ Alternate principal agency representative

Food and Drug Administration
Office of the Commissioner
* Suzanne Fitzpatrick, Ph.D., DABT
Center for Biologics Evaluation and Research
Richard McFarland, Ph.D., M.D.
Ying Huang, Ph.D.
Center for Devices and Radiological Health
Melvin E. Stratmeyer, Ph.D.
Vasant G. Malshet, Ph.D., DABT
Center for Drug Evaluation and Research
+ Abigail C. Jacobs, Ph.D.
Paul C. Brown, Ph.D.
Center for Food Safety and Applied Nutrition
David G. Hattan, Ph.D.
Robert L. Bronaugh, Ph.D.
Center for Veterinary Medicine
Devaraya Jagannath, Ph.D.
M. Cecilia Aguila, D.V.M.
National Center for Toxicological Research
Paul Howard, Ph.D.
Donna Mendrick, Ph.D.
William T. Allaben, Ph.D. (to Jan. 2009)
Office of Regulatory Affairs
Lawrence D'Hoostelaere, Ph.D.

National Cancer Institute
* T. Kevin Howcroft, Ph.D.
Chand Khanna, D.V.M., Ph.D.
Alan Poland, M.D. (to Oct. 2008)

National Institute of Environmental Health Sciences
* William S. Stokes, D.V.M., DACLAM
+ Raymond R. Tice, Ph.D.
Rajendra S. Chhabra, Ph.D., DABT
Jerrold J. Heindel, Ph.D.

National Institute for Occupational Safety and Health
* Paul Nicolaysen, V.M.D.
+ K. Murali Rao, M.D., Ph.D.

National Institutes of Health
* Margaret D. Snyder, Ph.D.

National Library of Medicine
* Pertti (Bert) Hakkinen, Ph.D.
+ Jeanne Goshorn, M.S.

Occupational Safety and Health Administration
* Surender Ahir, Ph.D.

Acknowledgements

Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Ocular Toxicity Working Group (OTWG)

U.S. Consumer Product Safety Commission
Marilyn L. Wind, Ph.D.
Adrienne Layton, Ph.D.

U.S. Department of Defense
Harry Salem, Ph.D.

U.S. Department of Transportation
Steve Hwang, Ph.D.

U.S. Environmental Protection Agency
Office of Pesticide Programs
Meta Bonner, Ph.D.
Jonathan Chen, Ph.D.
John R. "Jack" Fowle III, Ph.D., DABT
Masih Hashim, D.V.M., Ph.D.
Karen Hicks
Marianne Lewis
Debbie McCall
Timothy McMahon, Ph.D.
Mark Perry
John Redden
Jenny Tao, Ph.D.
Office of Research and Development
Andrew Geller, Ph.D.
Office of Science Coordination and Policy
Karen Hamernik, Ph.D.

U.S. Food and Drug Administration
Center for Drug Evaluation and Research
Paul Brown, Ph.D.
Wiley Chambers, M.D.
Abigail (Abby) Jacobs, Ph.D.
Jill Merrill, Ph.D., DABT (OTWG Chair)
Center for Food Safety and Applied Nutrition
Robert Bronaugh, Ph.D.
Donnie Lowther
Office of the Commissioner
Suzanne Fitzpatrick, Ph.D., DABT

National Institute of Environmental Health Sciences
Warren Casey, Ph.D., DABT
Mark F. Cesta, D.V.M, DACVP
Raymond (Buck) Grissom, Ph.D.
William Stokes, D.V.M., DACLAM

Occupational Safety and Health Administration
Surender Ahir, Ph.D.

European Centre for the Validation of Alternative Methods – Liaison
João Barroso, Ph.D.
Thomas Cole, Ph.D.
Valerie Zuang, Ph.D.

Japanese Center for the Validation of Alternative Methods – Liaison
Hajime Kojima, Ph.D.

National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

National Institute of Environmental Health Sciences

William Stokes, D.V.M., DACLAM
Director; Project Officer

Warren Casey, Ph.D., DABT
Deputy Director

Deborah McCarley
Special Assistant; Assistant Project Officer

NICEATM Support Contract Staff (Integrated Laboratory Systems [ILS], Inc.)

David Allen, Ph.D.
Jonathan Hamm, Ph.D.
Nelson Johnson
Brett Jones, Ph.D.
Elizabeth Lipscomb, Ph.D.
Linda Litchfield
Steven Morefield, M.D.
Catherine Sprankle
James Truax, M.A.
Linda Wilson

Statistical Consultant for ILS, Inc.

Joseph Haseman, Ph.D.

Additional Reviewers for the *In Vitro* Ocular Corrosion and Irritation Test Methods Background Review Documents

Chantra Eskes, Eng., Ph.D.
ECVAM
Ispra, Italy

Bob Guest
SafePharm Laboratories, Ltd.
Derby, United Kingdom

John Harbell, Ph.D.
Institute for In Vitro Sciences
Gaithersburg, Maryland

Joe Haseman, Ph.D.
Consultant
Raleigh, North Carolina

Penny Jones
Unilever Research
Sharnbrook, United Kingdom

Menk Prinsen
TNO Nutrition & Food Research Institute
The Netherlands

Horst Spielmann, Dr.med.
ZEBET
Berlin, Germany

Companies and Individuals that Provided *In Vitro* and/or *In Vivo* Data for the BCOP Background Review Document

ICCVAM and NICEATM gratefully acknowledge the following individuals and institutions that submitted data to NICEATM for the evaluation of alternative ocular safety testing methods and approaches

Access Business Group
Luann Potts
Tom Truszkowski
Washington, DC

Cosmetics, Toiletry, and Fragrance Association
Carol Eisenmann, Ph.D.
Washington, DC

ECVAM
Chantra Eskes, Ph.D.
Ispra, Italy

ExxonMobil Biomedical Sciences, Inc.
James Freeman, Ph.D.
Annandale, NJ

Institute for In Vitro Sciences, Inc.
John Harbell Ph.D.
Gaithersburg, MD

JohnsonDiversey, Inc.
John Hamilton, Ph.D.
Sarah Willems, B.S.
Sturdivant, WI

Johnson & Johnson Pharmaceutical R&D
Freddy van Goethem, Ph.D.
Phillipe Vanparys, Ph.D.
Beerse, Belgium

L'Oreal
Christine Van den Berghe, Ph.D.
Paris, France

Merck
Joseph Sina, Ph.D.
West Point, PA

S.C. Johnson & Son, Inc./JohnsonDiversey, Inc.
Nicole Cuellar, M.S.
Judith Swanson, B.S./B.A.
Racine, WI

Preface

Accidental contact with hazardous chemicals frequently causes eye injury and visual impairment. United States and international regulatory agencies currently use the Draize rabbit eye test (Draize et al. 1944) to identify potential ocular hazards associated with chemicals. The U.S. Consumer Product Safety Commission (CPSC), U.S. Environmental Protection Agency (EPA), U.S. Food and Drug Administration, and U.S. Occupational Safety and Health Administration have testing requirements and guidelines for assessing the ocular irritation potential of substances such as pesticides, household products, pharmaceuticals, cosmetics, and agricultural and industrial chemicals.

Although ocular safety assessment has clearly helped to protect consumers and workers, concerns have been raised about the humane aspects of the Draize rabbit eye test. Regulatory authorities have adopted various modifications that reduce the number of animals used and the potential pain and distress associated with the procedure. Significant progress has been made during the last decade. Now tests require only one to three rabbits, compared to six rabbits per test in the original protocol. Provisions have been added that allow for animals with severe lesions or discomfort to be humanely euthanized.

The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) previously evaluated the validation status of the bovine corneal opacity and permeability (BCOP), isolated chicken eye (ICE), isolated rabbit eye (IRE), and hen's egg test–chorioallantoic membrane (HET-CAM) test methods for the identification of ocular corrosives or severe (irreversible) ocular irritants. ICCVAM used the EPA (2003a), United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (UN 2007), and European Union (EU 2001) regulatory hazard classification systems. In ICCVAM's assessment, the performance of the BCOP and ICE test methods substantiated their use in testing some substances for regulatory hazard classification. The IRE and HET-CAM test methods lacked sufficient performance and/or sufficient data to substantiate their use for regulatory hazard classification.

ICCVAM recommended that the BCOP and ICE test methods should be used in a tiered-testing strategy in which positive substances can be classified as ocular corrosives or severe irritants without animal testing. In accordance with the ICCVAM Authorization Act of 2000 (Public Law 106-545), these recommendations were made available to the public and provided to U.S. Federal agencies for consideration in the *ICCVAM Test Method Evaluation Report – In Vitro Ocular Toxicity Test Methods for Identifying Severe Irritants and Corrosives* (ICCVAM 2006b). The ICCVAM recommendations were accepted by U.S. Federal agencies, and *in vitro* test methods may now be used instead of the Draize rabbit eye test for certain regulatory testing purposes.

ICCVAM is now reviewing the validation status of these *in vitro* test methods for identification of nonsevere ocular irritants (that is, those that induce reversible ocular damage [EPA Category II, III; EU Category R36, GHS Category 2A, 2B]) and substances not classified as irritants (GHS NC or Not Labeled, EPA Category IV, FHSA Not Labeled, or EU Not Labeled) according to the GHS (UN 2007), EPA (EPA 2003a), FHSA (FHSA 2005), and EU (EU 2001) classification systems. The Federal Hazardous Substances Act (FHSA) classification system (FHSA 2005) as defined in the "Test for Eye Irritants" (i.e., "Irritant" or Not Labeled [as an irritant]) and published in 16 CFR 1500.42 (CPSC 2003) is also provided in the current background review documents. The FHSA classification system was not used in the previous analyses of test methods used for the identification of severe ocular irritants or corrosives because the FHSA classification is limited to irritants and is not intended to identify corrosive substances or to differentiate between severe and nonsevere irritants.

Accordingly, the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the ICCVAM Ocular Toxicity Working Group (OTWG) prepared draft background review documents that summarize the current validation status of each test method based on published studies and other data and information submitted in response to a

June 7, 2007, *Federal Register* request (72 FR 31582, available at http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR_E7_10966.pdf). The background review documents form the basis for draft ICCVAM test method recommendations, which are provided in separate documents. Liaisons from the European Centre for the Validation of Alternative Methods and the Japanese Center for the Validation of Alternative Methods will provide input and contribute to the OTWG throughout the evaluation process.

An international independent scientific peer review panel (Panel) met in public session on May 19–21, 2009, to develop conclusions and recommendations on the *in vitro* BCOP, ICE, IRE, and HET-CAM test methods. The Panel included expert scientists nominated by the European Centre for the Validation of Alternative Methods and the Japanese Center for the Validation of Alternative Methods. We anticipate that these organizations can use the subsequent independent Panel report to deliberate and develop their own test method recommendations (ICCVAM Peer Review Panel Report [ICCVAM 2009] available to the public for comment on July 12, 2009). The Panel considered these background review documents and evaluated the extent to which the available information supports the draft ICCVAM test method recommendations.

ICCVAM provided the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) with the draft BRD and draft Test Method Evaluation Report, the Panel's report, and all public comments. SACATM discussed these at their June 25-26, 2009, meeting, where public stakeholders were given another opportunity to comment. After SACATM's meeting, ICCVAM considered the SACATM comments, the Panel report, and all public comments before finalizing the background review document and test method recommendations. These recommendations will be forwarded to Federal agencies for their consideration and acceptance decisions where appropriate.

We gratefully acknowledge the organizations and scientists who provided data and information for this document. We also acknowledge the efforts of those individuals who helped prepare this background review document, including the following staff from the NICEATM support contractor, Integrated Laboratory Systems, Inc.: David Allen, Jon Hamm, Nelson Johnson, Brett Jones, Elizabeth Lipscomb, Linda Litchfield, Steven Morefield, Gregory Moyer, Catherine Sprankle, and Jim Truax. We also thank the members of the ICCVAM Ocular Toxicity Working Group, chaired by Karen Hamernik, Ph.D. (U.S. EPA) and Jill Merrill, Ph.D. (U.S. Food and Drug Administration), and ICCVAM representatives who reviewed and commented on draft versions. We also thank Valerie Zuang, Ph.D., and Hajime Kojima, Ph.D., liaisons to the Ocular Toxicity Working Group from the European Centre for the Validation of Alternative Methods and the Japanese Center for the Validation of Alternative Methods, respectively, for their participation.

Marilyn Wind, Ph.D.
Deputy Associate Executive Director
Directorate for Health Sciences
U.S. Consumer Product Safety Commission
Chair, ICCVAM

William S. Stokes, D.V.M., DACLAM
Rear Admiral/Assistant Surgeon General, U.S. Public Health Service
Director, NICEATM
Executive Director, ICCVAM

Executive Summary

In October 2003, the U.S. Environmental Protection Agency (EPA) submitted to the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) a nomination requesting the evaluation of several activities related to reducing, refining, and replacing the use of rabbits in the current *in vivo* Draize rabbit eye test (69 FR 13859 [March 24, 2004]). In response to this nomination, ICCVAM evaluated the validation status of the bovine corneal opacity and permeability (BCOP), hen's egg test–chorioallantoic membrane (HET-CAM), isolated chicken eye (ICE), and isolated rabbit eye (IRE) test methods. To evaluate how well these test methods identify ocular corrosives and severe irritants, ICCVAM used the EPA (2003a), European Union (EU 2001), and United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (UN 2007) classification systems.

ICCVAM considered the performance of two of these *in vitro* test methods, the BCOP and the ICE, to be sufficient to support their use in testing certain types of substances for regulatory hazard classification. The IRE and HET-CAM test methods lacked sufficient performance and/or sufficient data to support their use for regulatory hazard classification. ICCVAM recommended that the BCOP and ICE test methods should be used in a tiered-testing strategy that would classify positive substances as ocular corrosives or severe irritants without animal testing. These recommendations were accepted by U.S. Federal agencies, and, as a result, *in vitro* test methods may now be used instead of conventional tests for certain regulatory testing purposes.

ICCVAM is now reviewing the validation status of these *in vitro* test methods to identify nonsevere ocular irritants (those that cause reversible ocular damage [EPA Category II and III; EU R36; GHS Category 2A and 2B]) and substances not classified as irritants (EPA Category IV; EU Not Labeled; GHS Not Classified) according to the EPA (2003a), EU (2001), and GHS (UN 2007) classification systems. The U.S. Federal Hazardous Substances Act (FHSA) classification system, which is based on the testing guidelines and associated criteria included in 16 CFR 1500.42 (CPSC 2003), is also included in these evaluations. The FHSA classification system was not used in the original analyses (ability of the test methods to identify ocular corrosives and severe irritants) because the FHSA ocular hazard category that is assigned based on results from the Draize rabbit eye test (Draize et al. 1944) does not distinguish between ocular corrosives and severe irritants and less severe irritants. For this reason, an evaluation to identify ocular corrosives and severe irritants using the FHSA classification system was not possible.

- Because the FHSA classification system (2005) is based on a sequential testing strategy that uses up to 18 animals, only a small percentage of the substances in the BCOP database would be classifiable if the FHSA criteria were strictly applied. To maximize the number of substances included in these analyses, "proportionality" criteria were applied for the purpose of assigning an FHSA classification to test results that would require additional testing according to the FHSA sequential testing strategy. These "proportionality" criteria (FHSA-20% and FHSA-67%) are as follows:
- FHSA-20% is based on the proportion of positive animals needed to identify a substance as an irritant using the FHSA sequential testing strategy, where 20% of the animals must demonstrate a positive response for a substance to be identified as an irritant. A substance tested using 3 to 6 animals would not be labeled if ≤1/6 animals were positive based on the FHSA criteria. The substance would be labeled as an irritant if there were ≥1 positive animal in a 3- to 5-animal test or ≥2 positive animals in a 6-animal test.
- FHSA-67% is based on the proportion of positive animals needed to identify a substance as an irritant using the "first test" of the FHSA sequential testing strategy, where 67% of the animals must demonstrate a positive response for a substance to be identified as an irritant. A substance tested using 3 to 6 animals would not be labeled as an irritant if ≤1/6 animals were positive based on the FHSA criteria. The substance would be labeled as an irritant if there

were ≥2/3, 3/4, 4/5, or 4/6 positive animals. If 1/3, 1/4, 2/4, 1/5, 2/5, 3/5, 2/6, or 3/6 animals were positive, further testing would be required.

Together, the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) and the ICCVAM Ocular Toxicity Working Group prepared draft background review documents (BRDs) that summarize the available data and information regarding the validity (usefulness and limitations) of each test method. This BRD summarizes all available information for the BCOP test method and its current validation status, including what is known about its reliability and accuracy, and the scope of the substances tested. Original data for the BCOP test method will be maintained for future use so that these performance statistics may be updated as additional information becomes available.

BCOP Test Method Protocol

The BCOP test method is an *in vitro* eye irritation test method that uses isolated bovine eyes that are byproducts from processing plants. Changes in corneal opacity and permeability are assessed as a measure of test substance damage. To determine opacity, the amount of light transmitted through the cornea is measured with an opacitometer. To determine permeability, the amount of sodium fluorescein dye that passes through all corneal cell layers is measured with a visible light spectrophotometer. Both permeability and opacity are used to calculate an *in vitro* irritancy score (IVIS) that is used to assign an *in vitro* irritancy classification, which predicts the potential of a test substance to cause *in vivo* ocular irritation.

Validation Database

An online literature search was conducted to support the initial evaluation of the validation status of the BCOP test method. The search identified four publications containing BCOP test method results. However, none of these publications included raw data or referenced *in vivo* data. Some of these publications also included data from earlier studies that were already in the validation database. ICCVAM received the BCOP test results for 66 antimicrobial cleaning products (AMCPs) in a submission that describes a non-animal approach for evaluating eye irritation potential and labeling requirements for AMCPs. The previous validation database for the BCOP test method (ICCVAM 2006a) was updated to include these results.

The updated BCOP validation database contains a total of 211 substances, including 135 commercial products or formulations. The most commonly tested chemical classes are alcohols, carboxylic acids, esters, formulations, heterocyclic compounds, hydrocarbons, ketones, and onium compounds. The formulations tested include hair shampoos, personal care cleansers, detergents, bleaches, insect repellents, petroleum products, and fabric softeners. The most commonly tested product classes are chemical/synthetic intermediates, cleaners, drugs/pharmaceuticals/therapeutic agents, petroleum products, solvents, shampoos, and surfactants.

In order to calculate the appropriate EPA (2003a), EU (2001), FHSA (2005), and GHS (UN 2007) ocular irritancy hazard classifications, detailed *in vivo* data consisting of cornea, iris, and conjunctiva scores for each animal at 24, 48, and 72 hours following test substance administration and/or assessment of the presence or absence of lesions at 7, 14, and 21 days are needed. Some of the test substances had only limited *in vivo* data and could not be used to evaluate test method accuracy and reliability. To maximize the number of substances included in the FHSA analyses, "proportionality" criteria (FHSA-20% and FHSA-67%), as outlined above, were applied for the purpose of assigning a FHSA classification to test results that would require additional testing according to the FHSA sequential testing strategy.

BCOP Test Method Accuracy

Identification of All Ocular Hazard Categories

ICCVAM evaluated how well the BCOP test method identified all categories of ocular irritation potential as defined by the EPA (2003a), GHS (UN 2007), and EU (2001) classification systems. Because the FHSA classification system does not distinguish between ocular corrosives and severe irritants and less severe irritants, an evaluation for all ocular hazard categories using the FHSA classification system was not possible.

As shown in **Table 1**, overall correct classifications ranged from 49% (91/187) to 55% (102/187) when using the entire database, depending on the hazard classification system used. Using different decision criteria to identify ocular corrosive/severe irritants (IVIS ≥ 75), based on the AMCP BRD (2008), instead of IVIS ≥ 55.1 as outlined in the ICCVAM BCOP BRD (2006a), does not improve test method performance.

Distinguishing Substances Not Labeled as Irritants from All Other Hazard Categories

ICCVAM also evaluated how well the BCOP test method distinguished substances not labeled as irritants (EPA Category IV, GHS Not Classified, EU Not Labeled, FHSA Not Labeled) from all other ocular hazard categories (EPA Categories I, II, III; GHS Categories 1, 2A, 2B; EU R41, R36; FHSA Irritant) as defined by the EPA (2003a), GHS (UN 2007), EU (2001), and FHSA (2005) classification systems. Analyses were also performed excluding specific chemical classes and/or physical properties that were previously identified as discordant in the BCOP test method (alcohols, ketones, and solids) relative to the *in vivo* hazard classification (ICCVAM 2006a).

As shown in **Table 2**, overall accuracy ranged from 64% (76/118) to 83% (148/179, 155/187, and 161/194), depending on the hazard classification system used. The lowest false negative rate (0% [0/97 and 0/54]) was noted for the GHS and EU classification systems, followed by 5% (8/147 and 6/132) for FHSA-20% and FHSA-67% criteria, and 6% (8/142) for the EPA classification system. Among the eight false negatives for the EPA classification system, all were EPA Category III substances based on Draize rabbit eye test data. For the FHSA-20% and FHSA-67% criteria, eight and six substances were false negatives, respectively. The lowest false positive rate (53% [24/45, 25/47, and 25/47]) was noted for the EPA, FHSA-20%, and FHSA-67% classification systems, followed by 66% (42/64) for the EU classification system, and 70% (63/90) for the GHS classification system. The exclusion of discordant classes had a minor effect or no effect on accuracy (ranged from 60% (39/65) to 82% (53/65) when discordant classes were removed versus 64% (76/118) to 83% (148/179, 155/187, and 161/194) for overall accuracy, depending on the hazard classification system used.

BCOP Test Method Reliability

Interlaboratory Reproducibility

Previous quantitative and qualitative evaluations of the reliability of the BCOP test method have been conducted (ICCVAM 2006a). Additional qualitative analyses of interlaboratory reproducibility were conducted to evaluate how well the BCOP hazard classifications agreed among the participating laboratories from the three different interlaboratory validation studies (Balls et al. 1995; Gautheron et al. 1994; Southee 1998). These evaluations were based on the use of the BCOP test method (1) to identify all ocular hazard categories according to the EPA, EU, or GHS systems, and (2) to distinguish substances not labeled as irritants (EPA Category IV, GHS Not Classified, EU Not Labeled) from all other ocular hazard categories (EPA Categories I, II, III; GHS Categories 1, 2A, 2B; EU R41, R36). Because the performance of the BCOP test method was similar for the EPA and FHSA hazard classification systems, additional reliability analyses were not conducted for the FHSA hazard classification system.

Table 1 Evaluation of the Performance of the BCOP Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by GHS, EPA, and EU Classification Systems[1]

Severe using IVIS ≥55.1											
Hazard Classification System	Overall Correct Classification	Severe[2]		Moderate[3]			Mild[4]			Not Labeled[5]	
		Actual	Under	Over	Actual	Under	Over	Actual	Under	Over	Actual
Severe using IVIS ≥ 55.1 (ICCVAM BCOP BRD [2006a])											
GHS	49% (91/187)	85% (55/65)	15% (10/65)	62% (16/26)	27% (7/26)	11% (3/26)	67% (4/6)	33% (2/6)	0% (0/6)	70% (63/90)	30% (27/90)
EPA	55% (102/187)	84% (53/63)	16% (10/63)	50% (11/22)	32% (7/22)	18% (4/22)	50% (28/57)	36% (21/57)	14% (8/57)	53% (24/45)	47% (21/45)
EU	50% (59/118)	79% (26/33)	21% (7/33)	48% (10/21)	52% (11/21)	0% (0/21)	NA	NA	NA	66% (42/64)	34% (22/64)
Severe using IVIS ≥75 (AMCP BRD [2008])											
Hazard Classification System		Severe		Moderate			Mild			Not Labeled	
		Actual	Under	Over	Actual	Under	Over	Actual	Under	Over	Actual
GHS	50% (94/187)	78% (51/65)	22% (14/65)	31% (8/26)	54% (14/26)	15% (4/26)	67% (4/6)	33% (2/6)	0% (0/6)	70% (63/90)	30% (27/90)
EPA	49% (92/187)	78% (49/63)	22% (14/63)	36% (8/22)	45% (10/22)	19% (4/22)	47% (27/57)	39% (22/57)	14% (8/57)	53% (24/45)	47% (21/45)
EU	51% (60/118)	73% (24/33)	27% (9/33)	29% (6/21)	67% (14/21)	4% (1/21)	NA	NA	NA	66% (42/64)	34% (22/64)

Abbreviations: BCOP = bovine corneal opacity and permeability; EPA = U.S. Environmental Protection Agency; EU = European Union; GHS = Globally Harmonized System; NA = not applicable.

[1] GHS classification system (UN 2007); EPA classification system (EPA 2003a); EU classification system (EU 2001). Because the FHSA classification system does not distinguish between ocular corrosives and severe irritants and less severe irritants, an evaluation for all ocular hazard categories using the FHSA classification system was not possible.

[2] Severe = EPA Category I; GHS Category 1; EU R41.

[3] Moderate = EPA Category II; GHS Category 2A; EU R36.

[4] Mild = EPA Category III; GHS Category 2B.

[5] Not Labeled = EPA Category IV; GHS Not Classified; EU Not Labeled.

Table 2 Accuracy of the BCOP Test Method in Distinguishing Substances Not Labeled as Irritants from All Other Irritant Classes, as Defined by the GHS, EPA, EU, and FHSA Classification Systems

Hazard Classification System	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
Overall (GHS)[1]	187	66	124/187	100	97/97	30	27/90	70	63/90	0	0/97
Without Alcohols, Ketones, and Solids[2]	66	64	42/66	100	34/34	25	8/32	75	24/32	0	0/34
Overall (EPA)[3]	187	83	155/187	94	134/142	47	21/45	53	24/45	6	8/142
Without Alcohols, Ketones, and Solids	65	82	53/65	96	47/49	44	7/16	56	9/16	4	2/49
Overall (EU)[4]	118	64	76/118	100	54/54	34	22/64	66	42/64	0	0/54
Without Alcohols, Ketones, and Solids	65	60	39/65	100	31/31	24	8/34	76	26/34	0	0/31
Overall (FHSA-20%)[5]	194	83	161/194	95	139/147	47	22/47	53	25/47	5	8/147
Without Alcohols, Ketones, and Solids	132	81	107/132	98	94/96	36	13/36	64	23/36	2	2/96
Overall (FHSA-67%)[5]	179	83	148/179	95	126/132	47	22/47	53	25/47	5	6/132
Without Alcohols, Ketones, and Solids	120	80	96/120	99	83/84	36	13/36	64	23/36	1	1/84

Abbreviations: BCOP = bovine corneal opacity and permeability; EPA = U.S. Environmental Protection Agency; EU = European Union; FHSA = Federal Hazardous Substances Act; GHS = Globally Harmonized System; N = number of substances included in this analysis; No. = data used to calculate the percentage.

[1] GHS classification system (UN 2007): Not Classified vs. Category 1/2A/2B.

[2] Alcohols, ketones, and solids were previously identified as discordant in the BCOP test method relative to the *in vivo* hazard classification (ICCVAM 2006a).

[3] EPA classification system (EPA 2003a): Category IV vs. Category I/II/III.

[4] EU classification system (EU 2001): Not Labeled vs. R41/R36.

[5] FHSA classification system (FHSA 2005): Not Labeled vs. Irritant. To maximize the number of substances included in the FHSA analyses, "proportionality" criteria (FHSA-20% and FHSA-67%) were applied for the purpose of assigning a FHSA classification to test results that would require additional testing according to the FHSA sequential testing strategy.

Using the first approach (identifying all ocular hazard categories) among the three interlaboratory studies for the Balls et al. (1995) study, there was 100% agreement among the five laboratories for a majority of the Draize ocular corrosives and severe irritants based on all three classification systems, whether they were correctly identified or underclassified by the BCOP test method. For example, for the GHS system, there was 100% agreement for 88% [15/17] of the correctly identified Category I substances. There was also 100% agreement among the five laboratories for 100% (10/10) of the overpredicted Not Labeled substances and for at least 50% (2/4) of the correctly identified Not Labeled substances.

For the Gautheron et al. (1994) study, there was 100% agreement among the 11 laboratories for a majority of the Draize ocular corrosives and severe irritants based on all three classification systems, whether they were correctly identified or underclassified by the BCOP test method. For example, for the GHS system, there was 100% agreement for 67% [4/6] of the correctly identified Category I substances. There was also 100% agreement among the 11 laboratories for a majority of the overpredicted Not Labeled substances (for example, for the EU system, there was 100% agreement for 54% [7/13] of the correctly identified Not Labeled substances) and for a majority of the incorrectly identified Not Labeled substances (for example, for the EU system, there was 100% agreement for 91% [21/23] of the correctly identified substances).

For the Southee (1998) study, there was 100% agreement among the three laboratories for all of the ocular corrosives and severe irritants based on all three classification systems, whether they were correctly identified or underclassified by the BCOP test method. For example, for the GHS system, there was 100% agreement for 100% [4/4] of the Draize ocular corrosives and severe irritants. There was also 100% agreement among the two correctly identified Not Labeled substances.

Using the second approach (distinguishing substances not labeled as irritants from all other ocular hazard categories) for the Balls et al. (1995) study, there was 100% agreement for 92% (55/60) to 93% (56/60) of the substances tested by the BCOP test method, depending on the classification system used. All five laboratories were in 100% agreement on the classification of 50% (2/4) of Not Labeled substances and 94% (32/34) to 96% (48/50) of all other irritant class substances, depending on the classification system used.

For the Gautheron et al. (1994) study, there was 100% agreement among the eleven laboratories for 65% (34/52) of the substances tested by the BCOP test method, for all classification systems. There was 100% agreement among the laboratories on the classification of 83% (10/12) to 87% (27/31) of all other irritant class substances, depending on the classification system used.

There was 100% agreement among the three laboratories in the Southee (1998) study for 88% (14/16) of the substances tested by the BCOP test method, for all classification systems. All three laboratories were in 100% agreement on the classification of 100% (2/2) Not Labeled substances and 90% (9/10) to 92% (11/12) of all other irritant class substances, depending on the classification system used.

1.0 Introduction

1.1 Background

The current Draize rabbit eye test method identifies both irreversible (i.e., corrosive) and reversible ocular effects. It also provides quantitative scoring with which to categorize the severity of reversible effects such as mild, moderate, or severe irritation. The current U.S. Environmental Protection Agency health effects test guideline for acute eye irritation (EPA 1998) and United Nations Globally Harmonized System (GHS) of Classification and Labelling of Chemicals (UN ocular testing strategy) indicate that if serious ocular damage is anticipated (e.g., a lesion considered to be irreversible or persisting for 21 days), then a test on a single animal may be considered. If serious damage is observed, no further animal testing is necessary (EPA 1998; UN 2007). If no serious damage is observed, additional test animals (1 or 2 rabbits) may be evaluated sequentially until concordant irritant or nonirritant responses are observed based on the GHS (UN 2007) or until unequivocal results are obtained in a minimum of three animals according to the EPA test guideline (EPA 1998). In the U.S. Federal Hazardous Substances Act (FHSA) classification system (FHSA 2005), which is based on the testing guidelines and associated criteria included in 16 CFR 1500.42 (CPSC 2003), corrosive substances are identified by other test methods (e.g., Draize skin test or human accidental exposure data) and excluded from further irritant testing.

In 2006, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) completed an evaluation of the bovine corneal opacity and permeability (BCOP) test method for its ability to identify ocular corrosives and severe irritants (ICCVAM 2006a). ICCVAM concluded that the BCOP test method could be used, in appropriate circumstances and with certain limitations, as a screening test to identify substances as ocular corrosives and severe irritants (i.e., EPA Category I, European Union [EU] R41, GHS Category 1) (ICCVAM 2006b). While it was not considered valid as a complete replacement for the *in vivo* rabbit eye test, the BCOP test method was recommended for use as part of a tiered-testing strategy for regulatory classification and labeling within a specific applicability domain. Accordingly, substances that are positive in this test method can be classified as ocular corrosives or severe irritants without further testing in rabbits, while a substance that tests negative would need additional testing in rabbits using a sequential testing strategy as outlined in Organisation for Economic Co-operation and Development Test Guideline 405 (OECD 2002).

ICCVAM is now evaluating the usefulness and limitations of the BCOP test method for identifying nonsevere irritants (i.e., those that induce reversible ocular damage [EPA Category II and III; EU R36; GHS Category 2A and 2B]) and substances not labeled as irritants (i.e., EPA Category IV; EU Not Labeled; FHSA Not Labeled; GHS Not Classified) according to the EPA, EU, FHSA, and GHS classification systems (EPA 2003a; EU 2001; FHSA 2005; UN 2007). However, because the FHSA classification system (2005) is based on a sequential testing strategy which uses up to 18 animals, only a small percentage of the substances in the BCOP database would be classifiable if the FHSA criteria were strictly applied. In order to maximize the number of substances included in these analyses, ”proportionality“ criteria (i.e., FHSA-20% and FHSA-67%) were applied for the purpose of assigning a FHSA classification for test results that would require additional testing according to the FHSA sequential testing strategy (see **Section 4.1**).

As part of the evaluation process, this background review document (BRD) has been prepared to describe the current validation status of the BCOP test method, including what is known about its reliability and accuracy, its applicability domain, the numbers and types of substances tested, and the availability of a standardized protocol. An ICCVAM expert panel used this BRD when reviewing the BCOP as a method to identify all categories of ocular irritants and substances not labeled as irritants.

Parallel reviews of the isolated rabbit eye (IRE), hen’s egg test–chorioallantoic membrane (HET-CAM), and isolated chicken eye (ICE) test methods are being conducted. The expert panel report and the

analyses presented in the BRDs will be used to support ICCVAM recommendations on the proposed standardized test method protocols, proposed list of recommended reference substances, and additional optimization and/or validation studies that may be necessary to further develop and characterize the usefulness and limitations of these methods.

For a more detailed discussion of the background of the BCOP test method, including its scientific basis and regulatory rationale and applicability, see the ICCVAM *Background Review Document—Current Status of In Vitro Test Methods for Identifying Ocular Corrosives and Severe Irritants: Bovine Corneal Opacity and Permeability* (ICCVAM 2006a).

1.2 Use of the BCOP Test Method in Overall Strategy of Hazard or Safety Assessment

As shown in **Figure 1-1**, the GHS allows for the use of validated and accepted *in vitro* methods to identify corrosive/severe ocular irritants and ocular irritants without further testing. The BCOP test method is currently recommended for use in identifying ocular corrosives and severe irritants in a tiered-testing strategy for regulatory classification and labeling (e.g., GHS, UN 2007). ICCVAM is now further evaluating the usefulness and limitations of the BCOP test method for identifying nonsevere irritants and substances not labeled as irritants.

1.3 Validation of the BCOP Test Method

The ICCVAM Authorization Act of 2000 (Sec. 4) mandates that "each Federal Agency … shall ensure that any new or revised … test method … is determined to be valid for its proposed use prior to requiring, recommending, or encouraging [its use]" (Public Law 106-545).

Validation is the process that establishes the reliability and relevance of a test method for a specific purpose (ICCVAM 2003). *Relevance* is defined as the extent to which a test method will correctly predict or measure the biological effect of interest (ICCVAM 2003). For the BCOP test method described in the BCOP BRD (ICCVAM 2006a), relevance is restricted to how well the test method identifies substances that are capable of producing corrosive or severe irritant effects on the eye. For the current BRD, relevance is based on how well the test method identifies (1) substances that are capable of producing nonsevere ocular irritation or (2) substances not labeled as irritants.

Reliability is defined as the reproducibility of a test method within and among laboratories. Reliability should be based on performance with a diverse set of substances that represent the types of chemical and product classes likely to be tested and that cover the range of responses that need to be identified. The validation process will provide data and information to allow U.S. Federal agencies to develop guidance on the development and use of the BCOP test method as part of a tiered-testing approach to evaluating substances' eye irritation potential.

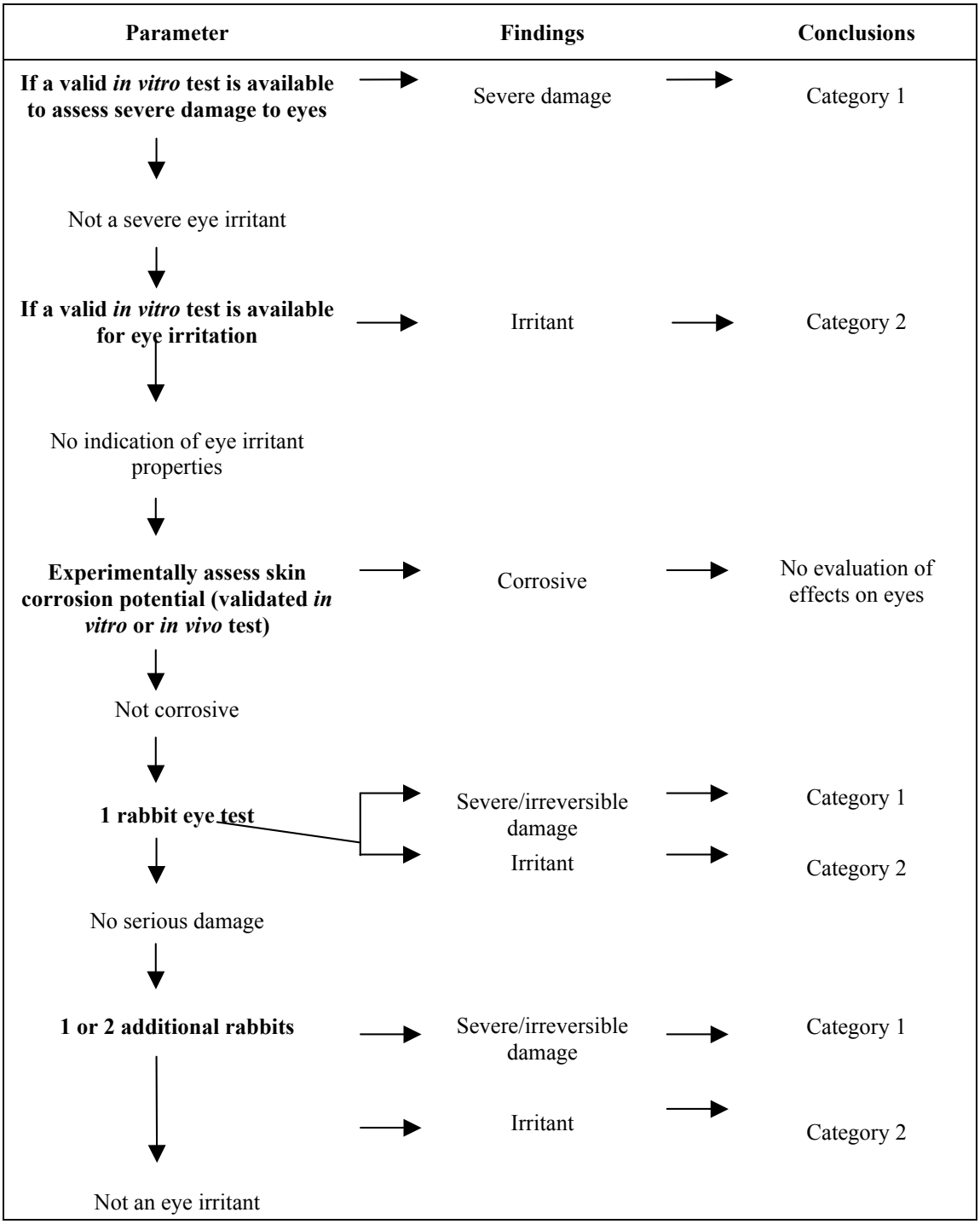
The first stage in this validation process is the preparation of a BRD that presents and evaluates the relevant data and information about the test method, including its mechanistic basis, proposed uses, reliability, and performance characteristics (ICCVAM 2003). This BRD summarizes the available information on the BCOP test method. Where adequate data is available, the qualitative and quantitative performance of the test method is evaluated.

1.4 Search Strategies and Selection of Citations for the BCOP BRD

The BCOP test method data summarized in this BRD are based on information found in the peer-reviewed scientific literature as detailed in the *Background Review Document—Current Status of In Vitro Test Methods for Identifying Ocular Corrosives and Severe Irritants: Bovine Corneal Opacity and Permeability Test Method* (ICCVAM 2006a). The National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) is currently evaluating a non-animal approach for assessing eye irritation potential and labeling requirements for antimicrobial cleaning

products (AMCPs). Three *in vitro* test methods, including the BCOP, are proposed in the testing strategy. The Institute for In Vitro Sciences gave the final AMCP BRD to NICEATM on July 21, 2008. Those substances in the AMCP validation database that had been tested in the BCOP test method were added to the BCOP validation database (ICCVAM 2006a). A subsequent literature search conducted in January 2009 revealed no new articles containing BCOP test method results.

Figure 1-1 GHS Testing Strategy for Serious Eye Damage and Eye Irritation



Adapted from UN (2007).

2.0 Bovine Corneal Opacity and Permeability Test Method Protocol Components

2.1 Overview of How the BCOP Test Method is Conducted

The BCOP test method is an *in vitro* model that provides short-term maintenance of the physiological and biochemical function of the bovine cornea. In this test method, damage by the test substance is assessed by quantitative measurements of changes in corneal opacity and permeability with an opacitometer and a visible light spectrophotometer, respectively. Both measurements are used to calculate an *in vitro* irritancy score (IVIS), which is used to assign an *in vitro* irritancy hazard classification category for prediction of the *in vivo* ocular irritation potential of a test substance.

For a detailed description of how the BCOP test method is conducted, see the *Background Review Document—Current Status of In Vitro Test Methods for Identifying Ocular Corrosives and Severe Irritants: Bovine Corneal Opacity and Permeability Test Method* (ICCVAM 2006a). Briefly, isolated corneas are obtained from the eyes of freshly slaughtered cattle. Test substances are applied to the epithelial surface of the cornea using different treatment methods depending on the physical nature and chemical characteristics (e.g., solids, semisolids [including creams and waxes], liquids, viscous [including gels] vs. nonviscous liquids) of the test substance. Liquids are tested undiluted, while surfactants are tested at a concentration of 10% in a 0.9% sodium chloride solution, distilled water, or other solvent demonstrated to have no adverse effects on the test system. Corneas are exposed to liquids and surfactants for 10 minutes. Nonsurfactant solids are typically tested as solutions or suspensions at a 20% concentration in a 0.9% sodium chloride solution, distilled water, or other solvent demonstrated to have no adverse effects on the test system. Solids may also be tested neat by direct application to the corneal surface. Corneas are exposed to solids for 4 hours.

Corneal opacity is quantified as the amount of light passing through the cornea, resulting in opacity values measured on a continuous scale. Permeability is quantified as the amount of sodium fluorescein dye that passes across the full thickness of the cornea, as detected in the posterior chamber medium. The mean opacity and mean permeability (OD_{490}) values for each treatment group are then used to calculate an *in vitro* score for each treatment group:

In vitro irritancy score = mean opacity value + (15 x mean OD490 value)

The *in vitro* irritation classification schemes used for this evaluation were based on two different predetermined ranges of *in vitro* scores. The differences between the two ranges are attributed to two different criteria used to identify ocular corrosives and severe irritants (i.e., EPA Category I, GHS Category 1, EU R41). One approach (**Table 2-1**) included the ICCVAM-recommended decision criteria for identifying an ocular corrosive/severe irritant (i.e., IVIS ≥ 55.1, ICCVAM 2006b).

Table 2-1 *In Vitro* Ocular Irritancy Classification Scheme for the BCOP Test Method (ICCVAM 2006b)

***In Vitro* Score Range**	***In Vitro* Classification**
0–3.0	Not Labeled
3.1–25	Mild Irritant
25.1–55	Moderate Irritant
≥55.1	Severe Irritant

The second approach (**Table 2-2**) included an alternative decision criterion for identifying an ocular corrosive/severe irritant in the AMCP BRD (2008) submission (i.e., IVIS ≥ 75).

Table 2-2 *In Vitro* Ocular Irritancy Classification Scheme for the BCOP Test Method (AMCP BRD 2008 Submission)

***In Vitro* Score Range**	***In Vitro* Classification**
0–3.0	Not Labeled
3.1–25	Mild Irritant
25.1–74.9	Moderate Irritant
≥75	Severe Irritant

For the purposes of this evaluation, Nonirritant = EPA Category IV, GHS Not Classified, EU Not Labeled, FHSA Not Labeled; Mild Irritant = EPA Category III, GHS Category 2B; Moderate Irritant = EPA Category II, GHS Category 2A; Severe Irritant = EPA Category I, GHS Category 1, EU Category R41. The Mild and Moderate Irritant categories were combined to generate EU Category R36. The Mild, Moderate, and Severe Irritant categories were combined to generate FHSA Irritant.

For this BRD, the *in vitro* classification was compared to the corresponding *in vivo* classification for each of the EPA, GHS, and EU classification systems (EPA 2003a; UN 2007; EU 2001). For the FHSA classification system, the *in vivo* classification was compared to the *in vitro* classification based on the EPA classification system. *In vitro* classifications of Mild, Moderate, and Severe Irritant were classified as FHSA Irritant and Nonirritant was classified as FHSA Not Labeled.

3.0 Substances Used for Validation of the Bovine Corneal Opacity and Permeability Test Method

In vitro ocular test method validation studies should evaluate an adequate sample of test substances and products from chemical and product classes that have also been evaluated using the *in vivo* rabbit eye test method. Test substances with a wide range of *in vivo* ocular responses (corrosive/severe irritant to Not Labeled) also should be assessed to determine limits to the range of responses that can be evaluated by the *in vitro* test method.

The substances tested in the BCOP test method and included in the AMCP BRD were added to BCOP data employed in the ICCVAM evaluation of the BCOP for identifying ocular corrosives and severe irritants (ICCVAM 2006a). Thus, the database in the current evaluation comprises substances from the AMCP BRD along with previously evaluated published reports (Bailey et al. 2004; Balls et al. 1995; Gautheron et al. 1994; Southee 1998; Swanson et al. 1995; Swanson and Harbell 2000).

Tables 3-1 and **3-2** show the chemical and product classes for the test substances included in the database. Information, including substance name, Chemical Abstracts Service Registry Number (CASRN), chemical and/or product class, concentration(s) tested, purity, supplier or source, and literature reference using the test substance are provided in **Annex I**. If not assigned in the study report, the product class was sought from other sources, including the National Library of Medicine's ChemIDplus® database. Chemical classes were assigned to each test substance using a standard classification scheme based on the National Library of Medicine Medical Subject Headings (MeSH®) classification system (available at http//www.nlm.nih.gov/mesh) that ensures consistency in classifying substances among all *in vitro* ocular test methods under consideration. A substance could be classified in more than one chemical or product class.

As shown in **Table 3-1**, the chemical classes with the greatest amount of *in vitro* BCOP data are alcohols, carboxylic acids, esters, formulations, heterocyclic compounds, hydrocarbons, ketones, and onium compounds. Other chemical classes tested include amines, ethers/polyethers, inorganic and organic salts, and organic sulfur compounds. The formulations tested include hair shampoos, personal care cleansers, detergents, bleaches, insect repellents, petroleum products, and fabric softeners.

As shown in **Table 3-2**, the product classes tested most often in the BCOP test method are AMCPs, chemical/synthetic intermediates, cleaners, drugs/pharmaceuticals/therapeutic agents, petroleum products, shampoos, solvents, and surfactants. Other product classes tested include detergents, insect repellents, lubricants, personal care cleansers, pesticides, and plasticizers.

Table 3-1 Chemical Classes Tested in the BCOP Test Method

Chemical Class	# of Substances	Chemical Class	# of Substances
Acyl halide	3	Imide	2
Alcohol	22	Inorganic salt	6
Aldehyde	1	Ketone	12
Alkali	3	Lactone	3
Aluminum compound	1	Nitrile compound	1
Amide	2	Nitro compound	2
Amidine	6	Oil	1
Amine	10	Onium compound	12
Amino acid	4	Organic salt	3
Boron compound	1	Organic sulfur compound	5
Carboxylic acid	17	Organophosphate	1
Ester	12	Organosilicon compound	1
Ether/Polyether	9	Phenol	1
Formulation	69	Polycyclic compound	3
Heterocyclic compound	12	Terpene	1
Hydrocarbon	18	Wax	1

Table 3-2 Product Classes Tested in the BCOP Test Method

Product Class	# of Substances	Product Class	# of Substances
Adhesive	1	Fertilizer	1
Agricultural chemical	2	Flame retardant	1
Antifreeze agent	1	Flavor ingredient	3
Antimicrobial cleaning product	66	Food additive	1
Bactericide/Fungicide/ Disinfectant/Germicide	11	Herbicide	3
Beverage	1	Insect repellant	8
Bleach	3	Lubricant/lubricant additive	6
Chelating agent	2	Paint, lacquer, varnish (component)	1
Chemical/synthetic intermediate	28	Pesticide	8
Cleaner	15	Petroleum product	16
Cleanser (personal care)	13	Photographic chemical/ developing agent	2
Coupling agent	1	Plant growth regulator	2
Cutting fluid	2	Plasticizer	4
Degreaser	1	Preservative	2
Dessicant	1	Reagent	5
Detergent	11	Shampoo (hair)	14
Drug/Pharmaceutical/ Therapeutic agent and/or metabolite	17	Soap	3
Dry cleaning preparation	1	Solvent	34
Dye, in manufacture of	3	Surfactant	39
Emulsifier	1	Anionic surfactant	3
Etching and/or electroplating	2	Cationic surfactant	6
Explosive	1	Nonionic surfactant	5
Fabric softener	1	Thermometer fluid	1

4.0 *In Vivo* Reference Data Used for an Assessment of Test Method Accuracy

The Draize rabbit eye test protocol used to generate the *in vivo* reference data is detailed in the ICCVAM *Test Method Evaluation Report: In Vitro Ocular Toxicity Methods for Identifying Severe Irritants and Corrosives* (2006b). A number of national and international test guidelines also describe this procedure (CPSC 2003; EPA 1998; EU 2004; OECD 2002). The subjective scoring system used to assign an ocular hazard classification is based on a discrete scale for grading the severity of ocular lesions on the cornea, iris, and conjunctiva.

Most of the BCOP studies evaluated in this BRD include *in vivo* reference data generated using the basic procedures for the Draize rabbit eye test method. NICEATM used these data to assign an ocular hazard classification according to the EPA (2003a), EU (2001), FHSA (2005), and the GHS (UN 2007) ocular irritancy classification systems (**Annex III**). Exceptions included the following:

For Gautheron et al. (1994), the *in vivo* reference data were obtained from concurrent *in vivo* studies performed by Dr. J. Giroux at the Agence du Medicament in Montpelier, France. Studies were performed according to European Economic Committee (EEC) (1984 and 1991) guidelines with a few modifications. Three rabbits were used per test substance, and a maximum average score (MAS) (Draize et al. 1944) was calculated. Only the MAS and Day 1 scores for the 52 compounds are presented in the Gautheron et al. publication. The substances were classified by the study authors according to both EEC (1984) and Kay and Calandra (1962) systems. Detailed *in vivo* data consisting of cornea, iris, and conjunctiva scores for each animal were provided by Dr. Philippe Vanparys in January 2005. Sufficient *in vivo* data were provided to allow 48 to 52 of these substances to be classified by NICEATM according to the EPA (EPA 2003a), EU (EU 2001) FHSA (2005), and GHS (UN 2007) ocular irritancy classification systems (**Annex III**).

For the European Commission/British Home Office validation study (Balls et al. 1995), modified maximum average scores (MMASs) were calculated for the 59 test substances from existing and concurrently run *in vivo* studies, all of which were performed according to OECD Test Guideline 405 and Good Laboratory Practices (GLP) guidelines. The data were generated since 1981 and met the following criteria:

- At least 3 New Zealand White rabbits were normally tested at the same time.
- A volume of 0.1 mL or the equivalent weight of substance was instilled into the conjunctival sac.
- Anesthesia was not used.
- Observations were made at least at 1, 2, and 3 days after instillation.

All 59 of these substances were classified by NICEATM according to the EU (2001) classification system, but due to lack of sufficient *in vivo* data, only 52, 55, 57, and 58 substances, respectively, were classified according to the FHSA-67% (2005), EPA (2003a), GHS (UN 2007), and the FHSA-20% ocular irritancy classification systems (**Annex III**).

For the Swanson et al. (1995) study, *in vivo* reference data were obtained from standard (100 μL of test material; 7 formulations) or modified (30 μL of test material; 13 formulations) Draize rabbit eye tests. An MAS(30) or an MAS(100) was reported for each test substance. *In vivo* categories reported in the publication are mild (2 substances), mild/moderate (2), moderate (4), moderate/severe (1), severe/corrosive (4), and corrosive (7). These categories are based on an internal classification scheme used at S.C. Johnson & Son, Inc. After publication of the study, the sponsor, S.C. Johnson & Son, Inc., assigned EPA (2003a) and GHS (UN 2007) classifications to the substances. The sponsor provided these classifications, along with detailed *in vivo* data for each test substance, to NICEATM. NICEATM verified the EPA and GHS ocular irritancy classifications for 13 of the substances and classified the

same 13 test substances based on the EU (2001) and FHSA (2005) ocular irritancy classification systems (**Annex III**). However, 11 of the test substances evaluated using a 30 µL test substance volume were not included in the accuracy analysis, because definitive classifications could not be assigned for the four regulatory ocular irritancy classification systems.

For the European Community prevalidation study of the BCOP test method (Southee 1998), cornea, iris, and conjunctiva scores for each animal for all substances were available in the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) Reference Chemicals data bank (ECETOC 1998). Fifteen of the substances have been classified by NICEATM according to the EU (2001) and FHSA-20% (2005) systems; 14 of the substances were classified according to the EPA (2003a, GHS (UN 2007) and the FHSA-67% (2005) ocular irritancy classification systems (**Annex III**).

S.C. Johnson and Son, Inc., provided detailed *in vivo* reference data for 9 of the 13 test substances evaluated in the Swanson and Harbell (2000) study of ethanol-containing insect repellent formulations. The standard Draize rabbit eye test protocol was used for these nine test substances. Each test included six animals.

ExxonMobil Biomedical Sciences, Inc., provided detailed *in vivo* reference data for the 16 petrochemical products evaluated by Bailey et al. (2004). All 16 substances had been tested previously using the standard Draize rabbit eye test protocol. Each test included either three or six animals.

4.1 *In Vivo* Classification Criteria Used for BRD Analysis

As described in the ICCVAM *Background Review Document—Current Status of In Vitro Test Methods for Identifying Ocular Corrosives and Severe Irritants: Bovine Corneal Opacity and Permeability Test Method* (2006a), the *in vivo* rabbit eye test database used to analyze the accuracy of the BCOP test method includes studies that were conducted using one to six rabbits. However, some of the *in vivo* classification systems considered for the accuracy analyses are designed to be applied to studies using no more than three rabbits. Thus, to maximize the amount of data used for the evaluation of the BCOP test method, the decision criteria for each classification system were expanded to include studies that used more than three rabbits. The criteria used for classification according to the EPA (2003a), EU (2001), and GHS (UN 2007) classification systems were detailed in the 2006 ICCVAM BRD. Each of these classification systems requires that the Draize scoring system be used. For these classification systems, scoring continues until the effect is cleared, but usually not beyond 21 days after the substance is applied to the eye of the rabbit. In order for a substance to have been included in the accuracy evaluations in the 2006 ICCVAM BRD, the following four criteria must have been met.

At least three rabbits were tested in the study unless a severe effect (e.g., corrosion of the cornea) was noted in a single rabbit. In such cases, substance classification could proceed based on the effects observed in less than three rabbits.

A volume of 0.1 mL or 0.1 g was tested in each rabbit. A study in which a lower quantity was applied to the eye was accepted for substance classification provided that a severe effect (e.g., corrosion of the cornea, lesion persistence) was observed in a rabbit.

Observations of the eye were made at least 24, 48, and 72 hours after test substance application if no severe effect was observed.

Observations of the eye were made until reversibility was assessed, typically meaning that all endpoint scores were cleared. Results from a study terminated early were not used unless the reason for the early termination was documented.

If any of the above criteria were not fulfilled, then the data for that substance were omitted from the accuracy analyses. The rules used for classification according to the EPA, EU, or GHS classification systems are detailed in the ICCVAM *Background Review Document—Current Status of In Vitro Test*

Methods for Identifying Ocular Corrosives and Severe Irritants: Bovine Corneal Opacity and Permeability Test Method (2006a).

For the FHSA classification system (FHSA 2005), the testing guidelines and associated criteria are included in 16 CFR 1500.42 (CPSC 2003). The FHSA classification system is based on using up to three sequential tests for each test substance with six animals used per test (**Table 4-1**). Decisions on further sequential testing are based on the number of positive responses in each test. The severity of effects for each endpoint (i.e., corneal ulceration and opacity, conjunctival redness and/or swelling, and iritis) is measured at 24, 48, and 72 hr following test substance administration. Positive responses include corneal ulceration (other than a fine stippling), corneal opacity or iritis ≥1, and conjunctival swelling and/or redness ≥2. In the first test, six animals are tested. If ≥4 animals are positive, the test is positive. If ≤1 animal tests positive, the test is negative. If 2/6 or 3/6 animals are positive, then a second test is performed with six additional animals. A third test is needed if 1/6 or 2/6 animals are positive with the second test.

The FHSA classification system (FHSA 2005) is a binary system, which classifies substances that test positive (according to the criteria provided in **Table 4-1)** as an irritant and substances that test negative as not requiring labeling (i.e. FHSA Not Labeled). Based on the FHSA sequential testing strategy, a substance can be classified as an eye irritant hazard with a few as 22% of the animals having a positive response (i.e., 2/6 [first test] +1/6 [second test] +1/6 [third test] = 4/18 or 22%).

Because the FHSA classification system is based on a sequential testing strategy, which uses up to 18 animals, only a small percentage of the substances in BCOP database would be classifiable if the FHSA criteria were strictly applied. In order to maximize the number of substances include in these analyses, "proportionality" criteria were developed by NICEATM for the purpose of assigning a FHSA classification for test results that would require additional testing according to the FHSA sequential strategy (**Table 4-2**).

Table 4-1 FHSA Classification System (16 CFR 1500.42)[1,2]

Positive Response for a Single Rabbit[3] **≥1 of the following at 24, 48, and/or 72 hr**	***In Vivo* Effect**
Corneal ulceration (other than a fine stippling) Corneal opacity (CO) ≥1 Iritis (IR) ≥1 Conjuctival redness (CR) and/or chemosis (CC) ≥2	First Test – If ≥4/6 animals are positive, the test is positive. If ≤1 animal is positive, the test is negative. If 2/6 or 3/6 animals are positive, the test is repeated using a different group of six animals. Second Test – If ≥3/6 animals are positive, the test is positive. If 0/6 animals are positive, the test is negative. If 1/6 or 2/6 animals are positive, the test is repeated using a different group of six animals. Third Test – Should a third test be needed, the test is positive if ≥1/6 animals are positive. If 0/6 animals are positive, the test is negative.

Abbreviations: CC = conjunctival chemosis; CFR = Code of Federal Regulations; CO = corneal opacity; CR = conjunctival redness; FHSA = Federal Hazardous Substances Act; IR = iritis

1 For the FHSA Classification System (2005), the testing guidelines and associated criteria are included in 16 CFR 1500.42 (CPSC 2003).

2 At least three animals per test (one animal screen for corrosive/severe irritants permitted). Maximum score in any animal used for classification.

3 The following scores are considered positive: CO or IR ≥1 or CR or CC ≥2. Therefore, CO and IR scores of 0 or CR and CC scores ≤1 are considered negative.

Table 4-2 Proposed FHSA "Proportionality" Criteria

No. of Animals in Test	FHSA-20%[1]		FHSA-67%[1]		
	NL	Irritant	NL	Irritant	Further Testing Required
3	0/3	≥1 (≥33%)	0/3	≥2 (≥67%)	1/3
4	0/4	≥1 (≥25%)	0/4	≥3 (≥75%)	1/4, 2/4
5	0/5	≥1 (≥20%)	0/5	≥4 (≥80%)	1/5, 2/5, 3/5
6	0/6, 1/6	≥2 (≥33%)	0/6, 1/6	≥4 (≥67%)	2/6, 3/6

Abbreviations: CPSC = U.S. Consumer Product Safety Commission; FHSA = Federal Hazardous Substances Act; NL = not labeled; No. = number

[1] FHSA-20% and FHSA-67% analysis methods are based on the proportionality of positive animals needed to identify a substance as an irritant.

[2] For FHSA-67%, Further Testing Required refers to substances that do not meet adequate positive or negative criteria to be classified.

These "proportionality" criteria (i.e., FHSA-20% and FHSA-67%) are as follows:

- (FHSA-20%) – FHSA-20% is based on the proportion of positive animals needed to identify a substance as an irritant using the FHSA sequential testing strategy, where 20% of the animals need to demonstrate a positive response for a substance to be identified as an irritant. A substance tested using 3 to 6 animals would not be labeled if ≤ 1/6 animals were positive based on the FHSA criteria. The substance would be labeled as an irritant if there were ≥1 positive animal in a 3 to 5 animal test or ≥2 positive animals in a 6 animal test.
- (FHSA-67%) – FHSA-67% is based on the proportion of positive animals needed to identify a substance as an irritant using the "first test" of the FHSA sequential testing strategy, where 67% of the animals need to demonstrate a positive response for a substance to be identified as an irritant. A substance tested using 3 to 6 animals would not be labeled if ≤ 1/6 animals were positive based on the FHSA criteria. The substance would be labeled as an irritant if there were ≥2/3, 3/4, 4/5, or 4/6 positive animals. If 1/3, 1/4, 2/4, 1/5, 2/5, 3/5, 2/6, or 3/6 animals were positive, further testing would be required.

4.2 *In Vivo* Data Quality

Ideally, all data supporting the validity of a test method should be obtained and reported from studies conducted in accordance with GLP guidelines, which are nationally and internationally recognized rules designed to produce high-quality laboratory data and records (EPA 2003b, 2003c; FDA 2003; OECD 1998). To ensure the integrity, reliability, and accountability of a study, these guidelines provide an internationally standardized approach for the conduct of studies, reporting requirements, archival of study data and records, and information about the test protocol.

Although an attempt was made, original study records could not be obtained for the *in vivo* rabbit eye studies used to provide the comparative data in the published BCOP validation reports. Therefore, the extent to which they complied with GLP guidelines is based on the information provided in the reports themselves. Balls et al. (1995) and Southee (1998) explicitly state that GLP guidelines were followed. For the Bailey et al. (2004) report, approximately half of the *in vivo* studies were conducted according to GLP guidelines, while GLP compliance was not explicitly stated for the remaining substances. For Gautheron et al. (1994), the *in vivo* studies were conducted according to EEC test guidelines (1984 and 1991), predecessors of the current EU test guideline for eye irritation. However, this information alone does not give enough information about GLP compliance. For the remaining reports (Swanson et al. 1995 and Swanson and Harbell 2000), the extent of GLP compliance is not known.

5.0 Bovine Corneal Opacity and Permeability Test Method Data and Results

Eight reports, seven published and one unpublished, were obtained for this evaluation and used for an accuracy analysis. Test method data were extracted from seven publications, data submissions, or study reports, including Gautheron et al. (1994), Balls et al. (1995), Swanson et al. (1995), Southee (1998), Swanson and Harbell (2000), Bailey et al. (2004), and the AMCP BRD (2008). The data were sufficient for an accuracy analysis of the BCOP test method for the identification of all categories of ocular irritation. As detailed in **Section 6.0**, the data were evaluated collectively and on a per-study basis.

5.1 Availability of Copies of Original Data Used to Evaluate the Accuracy and Reliability

NICEATM staff made several attempts to obtain original *in vitro* and *in vivo* data from BCOP test method studies. In addition, NICEATM requested original BCOP data and *in vivo* reference data from authors of published BCOP studies. As a result of these efforts, some original BCOP test method data (i.e., corrected opacity and OD_{490} values for individual corneas) were obtained. The European Centre for the Validation of Alternative Methods (ECVAM) provided corrected opacity and OD_{490} values in a written report for 16 substances evaluated in the European Community Prevalidation Study of the BCOP (Southee 1998).

Dr. Joseph Sina also submitted corrected opacity and OD_{490} values electronically for 43 compounds; however, corresponding *in vivo* reference data was not obtained. ECVAM subsequently provided the mean opacity values, mean permeability values, and mean *in vitro* scores obtained for the 59 substances evaluated in the Balls et al. (1995) study. Dr. Freddy Van Goethem provided a summary table and individual cornea data for 52 compounds tested in the EEC validation study (Gautheron et al. 1994). S.C. Johnson & Son, Inc., provided transformed BCOP data (mean opacity, permeability, and *in vitro* scores) for the Swanson et al. (1995) and Swanson and Harbell (2000) studies. ExxonMobil Biomedical Sciences, Inc., provided detailed study reports for the Bailey et al. (2004) study.

The majority of other published BCOP reports, which are discussed in **Section 9.0**, did not contain sufficient *in vitro* or *in vivo* data with which to conduct an accuracy analysis.

5.2 Description of the Statistical Approaches Used to Evaluate the Resulting Data

The BCOP studies included in the accuracy analysis in this document (**Section 6.0**) evaluated variability in the BCOP test method by calculating the mean (± SD) for the opacity values and the OD_{490} values for each treatment group and control group. The mean opacity and mean permeability (OD_{490}) values for each treatment group were then used to calculate an *in vitro* irritancy score for each treatment group as follows:

$$\textit{In vitro}\ \text{irritancy score} = \text{mean opacity value} + (15 \times \text{mean } OD_{490} \text{ value})$$

Sina et al. (1995) reported that this formula was derived empirically during in-house and interlaboratory studies. The data generated for a series of 36 compounds in a multilaboratory study were subjected to a multivariate analysis to determine the equation of best fit between *in vivo* and *in vitro* data. Analysis performed by scientists at two separate companies generated nearly identical derived equations. The *in vitro* irritancy score provides a numerical value that can be used to compare the relative irritancy of test substances.

The accuracy analysis in this document focused on evaluating the ability of the BCOP test method to identify ocular corrosives and severe irritants as defined by the EPA (2003a), EU (2001), and GHS (UN 2007) hazard classification schemes. The decision criteria applied to *in vitro* data to classify a test substance as a severe ocular irritant or a nonsevere ocular irritant (i.e., mild irritant, moderate irritant)

and/or Not Labeled are similar for the four BCOP test method protocols (Gautheron et al. 1994; Balls et al. 1995; Southee 1998; Bailey et al. 2004). The *in vitro* irritation classification scheme used in these studies is similar to the decision criteria first proposed by Gautheron et al. (1994), for which *in vitro* irritancy categories were based on predetermined ranges of *in vitro* scores (see **Section 2.0**).

5.3 Summary of Results

Where provided, the specific information extracted for each substance included its name, CASRN (if available), the concentration tested, the available BCOP data (e.g., mean opacity value, mean OD_{490} value, standard deviation, number of replicates, mean *in vitro* score), the *in vitro* irritation classification of the test substance (based on the *in vitro* irritation classification scheme applied or noted by the study author), and the literature reference. Other supporting information, such as the source, purity, and physicochemical characteristics of the test substances, was included to the extent this information was available. If not provided, the CASRN was obtained from various sources, including the National Library of Medicine's ChemIDplus® database. Chemical and product classes were assigned based on the MeSH classification system (available at http://www.nlm.nih.gov/mesh). **Annex I** provides information on the names, synonyms, CASRNs, and chemical/product class, where available, for each substance. **Annex II** contains the *in vitro* BCOP test method data sorted by reference and alphabetically by substance name.

5.4 Use of Coded Chemicals and Compliance with GLP Guidelines

Ideally, all data supporting the validity of a test method should be obtained and reported in accordance with GLP guidelines and with the use of coded chemicals (OECD 1998; EPA 2003b, 2003c; FDA 2003). The data quality was evaluated by reviewing the methods sections in literature references and submitted reports. The quality of data presented in the reviewed literature references can be evaluated to the extent this information was provided in the published reports. Based on the available information, the reports that stated that they had followed GLP guidelines or used data obtained according to GLP guidelines were Bailey et al. (2004), Balls et al. (1995), and Southee (1998). The extent of GLP compliance for Swanson et al. (1995) and Swanson and Harbell (2000) were not known. The reports that said they used coded chemicals were Gautheron et al. (1994), Balls et al. (1995), Swanson et al. (1995), Southee (1998), Swanson and Harbell (2000), and Bailey et al. (2004).

6.0 Bovine Corneal Opacity and Permeability Test Method Accuracy

A critical component of an ICCVAM evaluation of the validation status of a test method is an assessment of the accuracy of the proposed test method when compared to the current reference test method (ICCVAM 2003). This aspect of test method performance is typically evaluated by calculating:

Accuracy (concordance): the proportion of correct outcomes (positive and negative) of a test method

Sensitivity: the proportion of all positive substances that are classified as positive

Specificity: the proportion of all negative substances that are classified as negative

Positive predictivity: the proportion of correct positive responses among substances testing positive

Negative predictivity: the proportion of correct negative responses among substances testing negative

False positive rate: the proportion of all negative substances that are falsely identified as positive

False negative rate: the proportion of all positive substances that are falsely identified as negative

ICCVAM evaluated the ability of the BCOP test method to identify all categories of ocular irritation potential as defined by the EPA (EPA 2003), GHS (UN 2007), and EU (EU 2001) classification systems. Given that the FHSA classification system is used to identify eye irritants based on incidence and does not differentiate between irreversible (i.e., corrosive or severe) and reversible (i.e., nonsevere) ocular effects based on Draize rabbit eye test results, an evaluation for all ocular hazard categories using the FHSA classification system was not possible.

Analyses were also performed with specific chemical classes and/or physical properties excluded based on their previous identification as discordant in the BCOP test method (ICCVAM 2006a). These evaluations were conducted on the overall data set by combining results from the reports indicated in **Section 5.0** then assigning an overall ocular irritancy classification for each substance (**Annexes II** and **III**). When the same substance was evaluated in multiple laboratories, an overall BCOP classification was based on the majority classification among all of the studies. When there were equal numbers of different irritancy classifications for substances (e.g., two tests classified a substance as Not Labeled as Irritant, and two tests classified a substance as a mild irritant), the more severe irritancy classification was used for the overall classification of the substance (mild irritant, in this case).

The *in vitro* irritation classification schemes used for this evaluation were based on two different predetermined ranges of *in vitro* scores. The differences between the two ranges are attributed to two different criteria used to identify ocular corrosives and severe irritants (i.e., EPA Category I, GHS Category 1, EU R41). One approach (**Table 2-1**) included the ICCVAM-recommended decision criteria for identifying an ocular corrosive/severe irritant (i.e., IVIS ≥ 55.1, ICCVAM 2006b). The second approach (**Table 2-2**) included an alternative decision criteria for identifying an ocular corrosive/severe irritant in the AMCP BRD (2008) submission (i.e., IVIS ≥75).

6.1 Ability to Distinguish Ocular Corrosives and Severe Irritants from All Other Classes

The BCOP test method has been recommended previously for use in identifying ocular corrosives and severe irritants (i.e., EPA Category I, GHS Category 1, and EU R41; ICCVAM 2006b). The original ICCVAM evaluation of the BCOP test method was based on 145 substances. Overall accuracy rates were 79% (113/143) to 81% (119/147) depending on the hazard classification system evaluation (i.e., EPA, GHS, or EU). False positive rates were 19% (20/103) to 21% (22/103), and false negative rates were 16% (7/43) to 25% (10/40), also depending on the hazard classification system.

Because additional substances with sufficient BCOP and *in vivo* data were added to the BCOP test method validation database, this evaluation was repeated to verify similar performance. Based on the

current BCOP validation database, which has increased to 211 substances, overall accuracy is 77% (91/118) to 79% (147/186) depending on the hazard classification system evaluation (i.e., EPA, GHS, or EU). The false positive rate is 23% (29/124) to 24% (29/122), and false negative rates are 15% (10/65) to 21% (7/33) depending on the hazard classification system evaluation (**Table 6-1**).

Table 6-1 Accuracy of the BCOP Test Method in Distinguishing Ocular Corrosives/Severe Irritants from All Other Categories, as Defined by the EPA, GHS, and EU Classification Systems[1]

BCOP	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
GHS	187	79	148/187	85	55/65	76	93/122	24	29/122	15	10/65
EPA	187	79	148/187	84	53/63	77	95/124	23	29/124	16	10/63
EU	118	77	91/118	79	26/33	76	65/85	24	20/85	21	7/33

Abbreviations: BCOP= bovine corneal opacity and permeability; EPA = U.S. Environmental Protection Agency; EU = European Union; GHS = Globally Harmonized System; N = number of substances included in this analysis; No. = data used to calculate the percentage.

[1] EPA classification system (EPA 2003a); GHS classification system (UN 2007); EU classification system (EU 2001)

The following sections provide detailed analyses and results of the performance of the BCOP test method for each of the ocular hazard classification systems (i.e., EPA, GHS, and EU).

6.2 GHS Classification System: BCOP Test Method Accuracy

This accuracy evaluation used seven reports: Gautheron et al. (1994), Balls et al. (1995), Swanson et al. (1995), Southee (1998), Swanson and Harbell (2000), Bailey et al. (2004), and the AMCP BRD (2008) submission. These included BCOP data for 211 substances, 187 of which had sufficient *in vivo* data to be assigned an ocular irritancy classification according to the GHS classification system (UN 2007 (see **Annex III**). Among these studies, Gautheron et al. (1994), Balls et al. (1995), and Southee (1998) provided BCOP data for substances tested in multiple laboratories. Thus a consensus *in vitro* classification had to be assigned to each substance. Based on results from *in vivo* rabbit eye experiments, 35% (65/187) were classified as Category 1, 14% (26/187) were classified as Category 2A, 3% (6/187) were classified as Category 2B, and 48% (90/187) were classified as Not Classified as Irritant. Twenty-four substances could not be classified according to the GHS classification system due to the lack of adequate animal data.

6.2.1 Identification of Category 1 Substances (Ocular Corrosives/Severe Irritants)

The BCOP test method correctly identified 85% (55/65) and 78% (51/65) of the Category 1 substances using decision criteria of IVIS ≥55.1 and IVIS ≥75, respectively (**Table 6-2**). Among the Category 1 substances that were underpredicted by BCOP (based on IVIS ≥55.1), 9% (6/65) were classified as Category 2A, and 6% (4/65) were classified as Category 2B. Among the Category 1 substances that were underpredicted by the BCOP test method (based on IVIS ≥75), 15% (10/65) were classified as Category 2A and 6% (4/65) were classified as Category 2B.

6.2.2 Identification of Category 2A Substances (Moderate Ocular Irritants)

Of the 26 substances that could be evaluated, the BCOP test method correctly identified 27% (7/26) as moderate irritants, overpredicted 62% (16/26), and underpredicted 11% (3/26) using decision criteria defining ocular corrosives/severe irritants ≥55.1 (**Table 6-2**). Using decision criteria defining ocular

corrosives/severe irritants ≥75, the BCOP test method correctly identified 54% (14/26) as moderate irritants, overpredicted 31% (8/26), and underpredicted 15% (4/26) (**Table 6-2**).

6.2.3 Identification of Category 2B Substances (Mild Ocular Irritants)

Regardless of the decision criteria used to define ocular corrosives/severe irritants, of the six substances that could be evaluated, the BCOP test method correctly identified 33% (2/6) as mild irritants while overpredicting 67% (4/6) (**Table 6-2**).

6.2.4 Identification of Substances Not Classified as Irritant

Regardless of the decision criteria used to define ocular corrosives/severe irritants, of the 90 substances that could be evaluated, the BCOP test method correctly identified 30% (27/90) as Not Classified as Irritant while overpredicting 70% (63/90) (**Table 6-2**).

6.2.5 Overall Correct Classification

As indicated in **Table 6-2**, the use of the alternative decision criteria proposed in the AMCP BRD (2008), in which ocular corrosives/severe irritants ≥75, did not improve the overall performance of BCOP hazard classification. Therefore, the remaining analyses will present results utilizing the ICCVAM-recommended decision criteria for ocular corrosives/severe irritants (≥55.1). Overall, correct classification for the entire database of 187 substances was 49% (91/187) but ranged from 25% (2/8) to 60% (6/10 or 9/15) when each of the eight individual validation databases was evaluated (**Table 6-3**).

6.2.6 Ability to Distinguish Substances Not Classified as Irritant from All Other Classes

In addition to evaluating the ability of the BCOP test method to identify each individual ocular hazard category according to the GHS classification system, ICCVAM evaluated the ability of the BCOP test method to distinguish substances not classified as irritants from all other irritant classes. Using this approach for the 187 substances considered, the BCOP test method has an accuracy of 66% (124/187), a sensitivity of 100% (97/97), a specificity of 30% (27/90), a false positive rate of 70% (63/90) and a false negative rate of 0% (0/97) (**Table 6-4**).

As detailed below, the results from each individual study were also evaluated separately.

Gautheron et al. (1994): Based upon the *in vivo* rabbit data, 47 substances could be assigned a GHS classification. Based on these 47 substances, the BCOP test method has an accuracy of 55% (26/47), sensitivity of 100% (13/13), specificity of 38% (13/34), false positive rate of 62% (21/34), and a false negative rate of 0% (0/13) (**Table 6-4**).

Balls et al. (1995): Based upon the *in vivo* rabbit data, 54 substances could be assigned a GHS classification. Based on these 54 substances, the BCOP test method has an accuracy of 83% (45/54), sensitivity of 100% (40/40), specificity of 36% (5/14), false positive rate of 64% (9/14), and a false negative rate of 0% (0/40) (**Table 6-4**).

Swanson et al. (1995): Based upon the *in vivo* rabbit data, 10 substances could be assigned a GHS classification. Based on these 10 substances, the BCOP test method has an accuracy of 60% (6/10), sensitivity of 100% (6/6), specificity of 0% (0/4), false positive rate of 100% (4/4), and a false negative rate of 0% (0/6) (**Table 6-4**).

Table 6-2 Performance of the BCOP Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by EPA, GHS, and EU Classification Systems[1]

	Overall Correct Classification	Severe[2]		Moderate[3]			Mild[4]			Not Labeled[5]	
Severe using IVIS ≥ 55.1											
		Actual	**Under**	**Over**	**Actual**	**Under**	**Over**	**Actual**	**Under**	**Over**	**Actual**
GHS	49% (91/187)	85% (55/65)	15% (10/65)	62% (16/26)	27% (7/26)	11% (3/26)	67% (4/6)	33% (2/6)	0% (0/6)	70% (63/90)	30% (27/90)
EPA	55% (102/187)	84% (53/63)	16% (10/63)	50% (11/22)	32% (7/22)	18% (4/22)	50% (28/57)	36% (21/57)	14% (8/57)	53% (24/45)	47% (21/45)
EU	50% (59/118)	79% (26/33)	21% (7/33)	48% (10/21)	52% (11/21)	0% (0/21)	NA	NA	NA	66% (42/64)	34% (22/64)
Severe using IVIS ≥ 75											
		Severe		**Moderate**			**Mild**			**Not Labeled**	
		Actual	**Under**	**Over**	**Actual**	**Under**	**Over**	**Actual**	**Under**	**Over**	**Actual**
GHS	50% (94/187)	78% (51/65)	22% (14/65)	31% (8/26)	54% (14/26)	15% (4/26)	67% (4/6)	33% (2/6)	0% (0/6)	70% (63/90)	30% (27/90)
EPA	49% (92/187)	78% (49/63)	22% (14/63)	36% (8/22)	45% (10/22)	19% (4/22)	47% (27/57)	39% (22/57)	14% (8/57)	53% (24/45)	47% (21/45)
EU	51% (60/118)	73% (24/33)	27% (9/33)	29% (6/21)	67% (14/21)	4% (1/21)	NA	NA	NA	66% (42/64)	34% (22/64)

Abbreviations: BCOP = bovine corneal opacity and permeability; EPA = U.S. Environmental Protection Agency; EU = European Union; GHS = Globally Harmonized System; IVIS = *in vitro* irritancy score; NA = not applicable.

[1] EPA classification system (EPA 2003a); GHS classification system (UN 2007); EU classification system (EU 2001).

[2] Severe = EPA Category I, GHS Category 1, EU R41.

[3] Moderate = EPA Category II, GHS Category 2A, EU R36.

[4] Mild = EPA Category III, GHS Category 2B, EU R36.

[5] Not Labeled = Not Labeled or Classified as Irritant.

Table 6-3 Performance of the BCOP Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the GHS Classification System,[1] by Study and Overall

Data Source	Overall Correct Classification	Severe (Category 1)		Moderate (Category 2A)			Mild (Category 2B)			Not Classified[2]	
		Actual	Under	Over	Actual	Under	Over	Actual	Under	Over	Actual
Gautheron et al. (1994)	43% (20/47)	75% (6/8)	25% (2/8)	67% (2/3)	33% (1/3)	0% (0/3)	100% (2/2)	0% (0/2)	0% (0/2)	62% (21/34)	38% (13/34)
Balls et al. (1995)	50% (27/54)	73% (16/22)	27% (6/22)	57% (8/14)	29% (4/14)	14% (2/14)	50% (2/4)	50% (2/4)	0% (0/4)	64% (9/14)	36% (5/14)
Swanson et al. (1995)	60% (6/10)	100% (6/6)	0% (0/6)	0% (0/0)	0% (0/0)	0% (0/0)	0% (0/0)	0% (0/0)	0% (0/0)	100% (4/4)	0% (0/4)
Southee (1998)	60% (9/15)	57% (4/7)	43% (3/7)	33% (1/3)	67% (2/3)	0% (0/3)	50% (0/2)	50% (1/2)	0% (0/2)	33% (1/3)	67% (2/3)
Swanson and Harbell (2000)	25% (2/8)	100% (1/1)	0% (0/1)	50% (2/4)	25% (1/4)	25% (1/4)	0% (0/0)	0% (0/0)	0% (0/0)	100% (3/3)	0% (0/3)
Bailey et al. (2004)	43% (6/14)	67% (2/3)	33% (1/3)	0% (0/0)	0% (0/0)	0% (0/0)	100% (1/1)	0% (0/1)	0% (0/1)	60% (6/10)	40% (4/10)
AMCP BRD (2008)	51% (33/65)	93% (27/29)	7% (2/29)	86% (6/7)	14% (1/7)	0% (0/7)	0% (0/0)	0% (0/0)	0% (0/0)	83% (24/29)	17% (5/29)
Overall	49% (91/187)	85% (55/65)	15% (10/65)	62% (16/26)	27% (7/26)	11% (3/26)	67% (4/6)	33% (2/6)	0% (0/6)	70% (63/90)	30% (27/90)

Abbreviations: AMCP = antimicrobial cleaning product; BCOP = bovine corneal opacity and permeability; BRD = background review document; GHS = Globally Harmonized System.

[1] GHS classification system (UN 2007).

[2] Not Classified = Not Classified as Irritant.

Table 6-4 Accuracy of the BCOP Test Method in Distinguishing Substances Not Classified as Irritants from All Other Irritant Classes, as Defined by the GHS Classification System,[1] by Study and Overall

Data Source	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
Gautheron et al. (1994)	47	55	26/47	100	13/13	38	13/34	62	21/34	0	0/13
Balls et al. (1995)	54	83	45/54	100	40/40	36	5/14	64	9/14	0	0/40
Swanson et al. (1995)	10	60	6/10	100	6/6	0	0/4	100	4/4	0	0/6
Southee (1998)	15	93	14/15	100	12/12	67	2/3	33	1/3	0	0/12
Swanson and Harbell (2000)	8	63	5/8	100	5/5	0	0/3	100	3/3	0	0/5
Bailey et al. (2004)	14	57	8/14	100	4/4	40	4/10	60	6/10	0	0/4
AMCP BRD (2008)	65	63	41/65	100	36/36	17	5/29	83	24/29	0	0/36
Overall	187	66	124/187	100	97/97	30	27/90	70	63/90	0	0/97

Abbreviations: BCOP = bovine corneal opacity and permeability; GHS = Globally Harmonized System; N = number of substances included in this analysis; No. = data used to calculate the percentage.

[1] GHS (UN 2007): NL vs. Categories 1/2A/2B.

Southee (1998): Based upon the *in vivo* rabbit data, 15 substances could be assigned a GHS classification. Based on these 15 substances, the BCOP test method has an accuracy of 93% (14/15), sensitivity of 100% (12/12), specificity of 67% (2/3), false positive rate of 33% (1/3), and a false negative rate of 0% (0/12) (**Table 6-4**).

Swanson and Harbell (2000): Based upon the *in vivo* rabbit data, eight substances could be assigned a GHS classification. Based on these eight substances, the BCOP test method has an accuracy of 63% (5/8), sensitivity of 100% (5/5), specificity of 0% (0/3), false positive rate of 100% (3/3), and a false negative rate of 0% (0/5) (**Table 6-4**).

Bailey et al. (2004): Based upon the *in vivo* rabbit data, 14 substances could be assigned a GHS classification. Based on these 14 substances, the BCOP test method has an accuracy of 57% (8/14), sensitivity of 100% (4/4), specificity of 40% (4/10), false positive rate of 60% (6/10), and a false negative rate of 0% (0/4) (**Table 6-4**).

AMCP BRD (2008): Based upon the *in vivo* rabbit data, 65 substances could be assigned a GHS classification. Based on these 65 substances, the BCOP test method has an accuracy of 63% (41/65), sensitivity of 100% (36/36), specificity of 17% (5/29), false positive rate of 83% (24/29), and a false negative rate of 0% (0/36) (**Table 6-4**).

6.2.7 Discordant Results According to the GHS Classification System

In order to evaluate BCOP test method responses that disagreed with the *in vivo* hazard classification, several accuracy subanalyses were performed. These included specific classes of chemicals and certain properties of interest considered relevant to ocular toxicity testing (e.g., surfactants, physical form) with sufficiently robust numbers of substances (n ≥5).

Table 6-5 shows some notable trends in the performance of the BCOP test method among these subgroups of substances. The chemical classes of substances that the BCOP test method most consistently overpredicted according to the GHS classification system were alcohols and hydrocarbons. Of the 53 overpredicted substances, eight were alcohols and eight were hydrocarbons. Additional chemical classes represented among the overpredicted substances were carboxylic acids (6), heterocyclic compounds (4), and esters (4). Among the 23 substances labeled as surfactants, the BCOP test method overpredicted 22% (5/23).

Forty-four of the substances overpredicted by the BCOP test method were liquids, and nine were solids. Considering the proportion of the total available database, the BCOP test method appears more likely to overpredict liquids (90/122 or 74%) than solids (32/122 or 26%).

Alcohols (2) and carboxylic acids (2) were most often underpredicted (i.e., false negatives[1]) by the BCOP test method according to the GHS classification system (see **Annex III**). As can be seen in **Table 6-5**, the 16 irritant substances labeled as surfactants were rarely underpredicted by the BCOP test method (7% [1/14] Category 1 substances was underpredicted; none of the Category 2A or 2B substances were underpredicted).

With regard to physical form, six of the substances underpredicted by the BCOP test method were liquids and five were solids. Given the proportion of the total available database, the BCOP test method appears more likely to underpredict solids (32/122 or 26%) than liquids (90/122 or 74%).

Table 6-6 shows the effects on the BCOP test method performance statistics of excluding from the data set problematic classes (i.e., those which gave the most discordant results according to the GHS classification system) identified in the BCOP BRD (ICCVAM 2006a). In general, exclusion of alcohols, ketones, or solids individually resulted in small changes in the performance statistics. Slight increases in the overall correct classification were noted with the exclusion of problematic classes, with the highest correct classification, 51% (49/97), noted when alcohols and ketones were both excluded. The exclusion of problematic classes had little impact on the ability of the BCOP test method to identify substances not labeled as irritants (see **Table 6-7**). Accuracy was 68% (83/122) with the entire database but ranged from 64% to 69% when problematic classes or combinations were excluded.

In **Table 6-5**, hydrocarbons are noted as discordant when the BCOP test method was evaluated for its ability to identify all hazard categories. Among the 11 hydrocarbons in the validation database, 73% (8/11) were overpredicted by the BCOP test method (**Table 6-5**). Compared to the entire database, exclusion of hydrocarbons resulted in only modest improvements in overall correct classification (50% [55/111] versus 48% (58/122]) and identification of Not Labeled substances (38% [19/50] versus 36% [22/61]) (**Table 6-6**). Exclusion of hydrocarbons also resulted in modest improvement in overall performance in identifying Not Labeled substances (see **Table 6-7**). Accuracy increased from 68% (83/112) to 72% (80/111). The false positive rate decreased from 64% (39/61) to 62% (31/50), while the false negative rate remained 0% (0/61 versus 0/61).

---

[1] *False negative* in this context refers to a substance that the BCOP test method classified as a nonsevere (mild or moderate) irritant or Not Labeled but that the *in vivo* data classified as a severe irritant.

Table 6-5 Under- and Overprediction of the BCOP Test Method Using the GHS Classification System[1] in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method by Chemical Class or Physical Property

Category	N	Underprediction (*In Vivo*/*In Vitro*)						Overprediction (*In Vivo*/*In Vitro*)					
		Severe (Category 1)			Moderate (Category 2A)		Mild (Cat 2B)	Moderate (Cat 2A)	Mild (Category 2B)		Not Classified as Irritant (NL)		
		2A	2B	NL	2B	NL	NL	1	1	2A	1	2A	2B
Overall	147	11% (4/36)	11% (4/36)	0% (0/36)	16% (3/19)	0% (0/19)	0% (0/6)	53% (10/19)	17% (1/6)	50% (3/6)	15% (9/61)	11% (7/61)	38% (23/61)
Chemical Class[2]													
Alcohol	18	33% (1/3)	33% (1/3)	0% (0/3)	0% (0/6)	0% (0/6)	0% (0/1)	67% (4/6)	0% (0/1)	100% (1/1)	43% (3/7)	0% (0/7)	0% (0/7)
Amine/Amidine	7	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/2)	0% (0/2)	0/0	0/0	0/0	0/0	0% (0/4)	0% (0/4)	25% (1/4)
Carboxylic acid	14	0% (0/6)	33% (2/6)	0% (0/6)	0% (0/2)	0% (0/2)	0/0	50% (1/2)	0/0	0/0	33% (2/6)	33% (2/6)	17% (1/6)
Ester	10	0% (0/2)	0% (0/2)	0% (0/2)	33% (1/3)	0% (0/3)	0% (0/1)	33% (1/3)	0% (0/1)	0% (0/1)	0% (0/4)	5% (2/4)	25% (1/4)
Ether	6	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)	0/0	100% (1/1)	0/0	0/0	25% (1/4)	0% (0/4)	0% (0/4)
Heterocyclic	13	0% (0/6)	17% (1/6)	0% (0/6)	0% (0/1)	0% (0/1)	0/0	0% (0/1)	0/0	0/0	17% (1/6)	0% (0/6)	50% (3/6)
Hydrocarbon	11	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	9% (1/11)	18% (2/11)	45% (5/11)
Inorganics	7	0% (0/4)	0% (0/4)	0% (0/4)	0/0	0/0	0% (0/1)	0/0	0% (0/1)	0% (0/1)	0% (0/2)	0% (0/2)	50% (1/2)
Ketone	9	0/0	0/0	0/0	0% (0/2)	0% (0/2)	0% (0/1)	0% (0/2)	0% (0/1)	0% (0/1)	33% (2/6)	0% (0/6)	17% (1/6)

Category	N	Underprediction (*In Vivo*/*In Vitro*)						Overprediction (*In Vivo*/*In Vitro*)					
		Severe (Category 1)			Moderate (Category 2A)		Mild (Cat 2B)	Moderate (Cat 2A)	Mild (Category 2B)		Not Classified as Irritant (NL)		
		2A	2B	NL	2B	NL	NL	1	1	2A	1	2A	2B
Onium compound	11	13% (1/8)	0% (0/8)	0% (0/8)	0/0	0/0	0% (0/1)	0/0	0% (0/1)	0% (0/1)	0% (0/2)	0% (0/2)	50% (1/2)
Polyether	2	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0% (0/2)	0% (0/2)	0% (0/2)
Properties of Interest													
Liquids	90	8% (2/24)	4% (1/24)	0% (0/24)	18% (3/17)	0% (0/17)	0% (0/5)	53% (9/17)	20% (1/5)	60% (3/5)	16% (7/44)	16% (7/44)	39% (17/44)
Solids	32	17% (2/12)	25% (3/12)	0% (0/12)	0% (0/2)	0% (0/2)	0/0	50% (1/2)	0/0	0/0	12% (2/17)	0% (0/17)	35% (6/17)
Pesticide	8	20% (1/5)	20% (1/5)	0% (0/5)	0% (0/1)	0% (0/1)	0/0	100% (1/1)	0/0	0/0	50% (1/2)	0% (0/2)	50% (1/2)
Surfactant-Total	23	0% (0/14)	7% (1/14)	0% (0/14)	0% (0/1)	0% (0/1)	0% (0/1)	100% (1/1)	0% (0/1)	0% (0/1)	0% (0/7)	14% (1/7)	43% (3/7)
-nonionic	10	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/1)	0% (0/1)	0/0	100% (1/1)	0/0	0/0	0% (0/4)	0% (0/4)	0% (0/4)
-anionic	9	0% (0/5)	20% (1/5)	0% (0/5)	0/0	0/0	0% (0/1)	0/0	0% (0/1)	0% (0/1)	0% (0/3)	33% (1/3)	67% (2/3)
-cationic	7	0% (0/6)	0% (0/6)	0% (0/6)	0/0	0/0	0/0	0/0	0/0	0/0	0% (0/1)	0% (0/1)	100% (1/1)

Abbreviations: BCOP = bovine corneal opacity and permeability; GHS = Globally Harmonized System.

[1] GHS classification system (UN 2007).

[2] Chemical classes included in this table are represented by at least five substances tested in the BCOP test method and assignments are based upon National Library of Medicine medical subject heading (MeSH) categories (www.nlm.nih.gov/mesh) as defined in Annex I.

Table 6-6 Performance of the BCOP Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the GHS Classification System,[1] with Discordant Chemical and Physical Classes Excluded

BCOP	Overall Correct Classification	Severe (Category 1)		Moderate (Category 2A)			Mild (Category 2B)			Not Classified[2]	
		Actual	Under	Over	Actual	Under	Over	Actual	Under	Over	Actual
Overall	48% (58/122)	78% (28/36)	22% (8/36)	53% (10/19)	32% (6/19)	15% (3/19)	67% (4/6)	33% (2/6)	0% (0/6)	64% (39/61)	36% (22/61)
Without Alcohols	49% (52/106)	82% (27/33)	18% (6/33)	46% (6/13)	31% (4/13)	23% (3/13)	60% (3/5)	40% (2/5)	0% (0/5)	65% (36/55)	35% (19/55)
Without Ketones	49% (55/113)	78% (28/36)	22% (8/36)	47% (8/17)	35% (6/17)	18% (3/17)	80% (4/5)	20% (1/5)	0% (0/5)	64% (35/55)	36% (20/55)
Without Solids	44% (40/90)	88% (21/24)	13% (3/24)	53% (9/17)	29% (5/17)	18% (3/17)	80% (4/5)	20% (1/5)	0% (0/5)	70% (31/44)	30% (13/44)
Without Alcohols and Ketones	51% (49/97)	82% (27/33)	18% (6/33)	36% (4/11)	36% (4/11)	27% (3/11)	75% (3/4)	25% (1/4)	0% (0/4)	65% (32/49)	35% (17/49)
Without Alcohols, Ketones, and Solids	47% (31/66)	91% (20/22)	9% (2/22)	33% (3/9)	34% (3/9)	33% (3/9)	100% (3/3)	0% (0/3)	0% (0/3)	75% (24/32)	25% (8/32)
Without Hydrocarbons	50% (55/111)	78% (28/36)	22% (8/36)	53% (10/19)	32% (6/19)	15% (3/19)	67% (4/6)	33% (2/6)	0% (0/6)	62% (31/50)	38% (19/50)

Abbreviations: BCOP = bovine corneal opacity and permeability; GHS = Globally Harmonized System.

[1] GHS classification system (UN 2007).

[2] Not Classified = Not Classified as Irritant.

Table 6-7 Accuracy of the BCOP Test Method in Distinguishing Substances Not Classified as Irritants from All Other Irritant Classes, as Defined by the GHS Classification System,[1] with Discordant Chemical and Physical Classes Excluded

BCOP	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
Overall	187	66	124/187	100	97/97	30	27/90	70	63/90	0	0/97
Without Alcohols	106	66	70/106	100	51/51	35	19/55	65	36/55	0	0/51
Without Ketones	113	69	78/113	100	58/58	36	20/55	64	65/55	0	0/58
Without Solids	90	66	59/90	100	46/46	30	13/44	70	31/44	0	0/46
Without Alcohols and Ketones	97	67	65/97	100	48/48	35	17/49	65	32/49	0	0/48
Without Alcohols, Ketones, and Solids	66	64	42/66	100	34/34	25	8/32	75	24/32	0	0/34
Without Hydrocarbons	111	72	80/111	100	61/61	38	19/50	62	31/50	0	0/61

Abbreviations: BCOP = bovine corneal opacity and permeability; GHS = Globally Harmonized System; N = number of substances included in this analysis/the total number of substances in the study; NL = Not Labeled; No. = data used to calculate the percentage.

[1] GHS classification system (UN 2007): NL vs. Categories 1/2A/2B.

6.3 EPA Classification System: BCOP Test Method Accuracy

The seven reports used in the accuracy evaluation (Gautheron et al. 1994; Balls et al. 1995 ; Swanson et al. 1995 ; Southee 1998; Swanson and Harbell 2000; Bailey et al. 2004; and the AMCP BRD 2008) included BCOP data on 211 substances, 187 of which had sufficient *in vivo* data to be assigned an ocular irritancy classification according to the EPA classification system (EPA 2003a) (see **Annex III**). Among these studies, Gautheron et al. (1994), Balls et al. (1995), and Southee (1998) provided BCOP data for substances tested in multiple laboratories and thus required that a consensus *in vitro* classification be assigned to each substance. Based on results from *in vivo* rabbit eye experiments, 35% (65/187) were classified as Category I, 14% (26/187) were classified as Category II, 3% (6/187) were classified as Category III, and 48% (90/187) were classified as Category IV. Twenty-four substances could not be classified according to the GHS classification system due to the lack of adequate animal data (noted in **Annex III)**.

6.3.1 Identification of Category I Substances (Ocular Corrosives/Severe Irritants)

The BCOP test method correctly identified 84% (53/63) and 78% (49/63) of the Category I substances using decision criteria defining ocular corrosives/severe irritants ≥55.1 and ocular corrosives/severe irritants ≥75, respectively (**Table 6-2**). Using decision criteria defining *in vitro* scores ≥55.1 as ocular corrosives/severe irritants, of the Category I substances that were underpredicted by the BCOP test method, 10% (6/63) were classified as Category II, and 6% (4/63) were classified as Category III. Using decision criteria defining *in vitro* scores ≥75 as ocular corrosives/severe irritants, of the Category I substances that were underpredicted by BCOP test method, 16% (10/63) were classified as Category II, and 6% (4/63) were classified as Category III.

6.3.2 Identification of Category II Substances (Moderate Ocular Irritants)

Of the 22 substances that could be evaluated, the BCOP test method correctly identified 32% (7/22) as moderate irritants, while 50% (11/22) were overpredicted and 18% (4/22) were underpredicted using decision criteria that defined *in vitro* scores ≥55.1 as ocular corrosives/severe irritants (**Table 6-8**). Using decision criteria defining *in vitro* scores ≥75 as ocular corrosives/severe irritants, the BCOP test method correctly identified 45% (10/22) as moderate irritants, while overpredicting 36% (8/22) and underpredicting 19% (4/22) (**Table 6-2**).

6.3.3 Identification of Category III Substances (Mild Ocular Irritants)

Using decision criteria defining *in vitro* scores ≥55.1 as ocular corrosives/severe irritants, for the 56 substances that could be evaluated, the BCOP test method correctly identified 36% (21/57) as mild irritants, while 50% (28/57) were overpredicted and 14% (8/57) were underpredicted (**Table 6-8**). Using decision criteria defining *in vitro* scores ≥75 as ocular corrosives/severe irritants, for the 57 substances that could be evaluated, the BCOP test method correctly identified 39% (22/57) as mild irritants, while 47% (27/57) were overpredicted and 14% (8/57) were underpredicted (**Table 6-2**).

6.3.4 Identification of Category IV Substances

Regardless of the decision criteria used to define *in vitro* scores as ocular corrosives/severe irritants, for the 45 substances that could be evaluated, the BCOP test method correctly identified 47% (21/45) as Category IV, while 53% (24/45) were overpredicted (**Tables 6-2 and 6-8**).

6.3.5 Ability to Distinguish Category IV from All Other Classes

In addition to evaluating the ability of the BCOP test method to identify each individual ocular hazard category according to the EPA classification system, ICCVAM also evaluated the ability of the BCOP

test method to distinguish Category IV from all other irritant classes. Using this approach for the 187 substances considered, the BCOP test method has an accuracy of 83% (155/187), a sensitivity of 94% (134/142), a specificity of 47% (21/45), a false positive rate of 53% (24/45), and a false negative rate of 6% (8/142) (**Table 6-9**).

As detailed below, the results from each individual study were also evaluated separately.

Gautheron et al. (1994): Based upon the *in vivo* rabbit data, 48 substances could be assigned an EPA classification. Based on these 48 substances, the BCOP test method has an accuracy of 83% (40/48), sensitivity of 89% (31/35), specificity of 69% (9/13), false positive rate of 31% (4/13), and a false negative rate of 11% (4/35) (**Table 6-9**).

Balls et al. (1995): Based upon the *in vivo* rabbit data, 54 substances could be assigned an EPA classification. Based on these 54 substances, the BCOP test method has an accuracy of 93% (50/54), sensitivity of 92% (48/52), specificity of 100% (2/2), false positive rate of 0% (0/2), and a false negative rate of 8% (4/52) (**Table 6-9**).

Swanson et al. (1995): Based upon the *in vivo* rabbit data 10 substances could be assigned an EPA classification. Based on these 10 substances, the BCOP test method has an accuracy of 90% (9/10), sensitivity of 100% (9/9), specificity of 0% (0/1), false positive rate of 100% (1/1), and a false negative rate of 0% (0/9) (**Table 6-9**).

Southee (1998): Based upon the *in vivo* rabbit data, 15 substances could be assigned an EPA classification. Based on these 15 substances, the BCOP test method has an accuracy of 93% (14/15), sensitivity of 93% (13/14), specificity of 100% (1/1), false positive rate of 0% (0/1), and a false negative rate of 7% (0/14) (**Table 6-9**).

Swanson and Harbell (2000): Based upon the *in vivo* rabbit data, eight substances could be assigned an EPA classification. Based on these eight substances, the BCOP test method has an accuracy of 75% (6/8), sensitivity of 100% (6/6), specificity of 0% (0/2), false positive rate of 100% (2/2), and a false negative rate of 0% (0/6) (**Table 6-9**).

Bailey et al. (2004): Based upon the *in vivo* rabbit data, 13 substances could be assigned an EPA classification. Based on these 13 substances, the BCOP test method has an accuracy of 62% (8/13), sensitivity of 75% (3/4), specificity of 44% (4/9), false positive rate of 56% (5/9), and a false negative rate of 25% (1/4) (**Table 6-9**).

AMCP BRD (2008): Based upon the *in vivo* rabbit data, 66 substances could be assigned an EPA classification. Based on these 66 substances, the BCOP test method has an accuracy of 79% (52/66), sensitivity of 98% (47/48), specificity of 28% (5/18), false positive rate of 72% (13/18), and a false negative rate of 2% (1/48) (**Table 6-9**).

Table 6-8 Performance of the BCOP Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the EPA Classification System,[1] by Study and Overall

Data Source	Overall Correct Classification	Severe (Category I)		Moderate (Category II)			Mild (Category III)			Not Labeled[2] (Category IV)	
		Actual	Under	Over	Actual	Under	Over	Actual	Under	Over	Actual
Gautheron et al. (1994)	52% (25/48)	75% (6/8)	25% (2/8)	75% (3/4)	25% (1/4)	0% (0/4)	44% (10/23)	39% (9/23)	17% (4/23)	31% (4/13)	69% (9/13)
Balls et al. (1995)	46% (25/54)	68% (13/19)	32% (6/19)	50% (6/12)	33% (4/12)	17% (2/12)	52% (11/21)	29% (6/21)	19% (4/21)	0% (0/2)	100% (2/2)
Swanson et al. (1995)	60% (6/10)	100% (6/6)	0% (0/6)	0% (0/0)	0% (0/0)	0% (0/0)	100% (3/3)	0% (0/3)	0% (0/3)	100% (0/1)	0% (0/1)
Southee (1998)	47% (7/15)	50% (3/6)	50% (3/6)	50% (1/2)	50% (1/2)	0% (0/2)	50% (3/6)	33% (2/6)	17% (1/6)	0% (0/1)	100% (1/1)
Swanson and Harbell (2000)	50% (4/8)	100% (3/3)	0% (0/3)	0% (0/2)	50% (1/2)	50% (1/2)	100% (1/1)	0% (0/1)	0% (0/1)	100% (2/2)	0% (0/2)
Bailey et al. (2004)	38% (5/13)	0% (0/1)	100% (1/1)	0% (0/0)	0% (0/0)	0% (0/0)	33% (1/3)	33% (1/3)	33% (1/3)	56% (5/9)	44% (4/9)
AMCP BRD (2008)	62% (41/66)	94% (29/31)	6% (2/31)	60% (3/5)	20% (1/5)	20% (1/5)	42% (5/12)	50% (6/12)	8% (1/12)	72% (13/18)	28% (5/18)
Overall	55% (102/187)	84% (53/63)	16% (10/63)	50% (11/22)	32% (7/22)	18% (4/22)	50% (28/57)	36% (21/57)	14% (8/57)	53% (24/45)	47% (21/45)

Abbreviations: AMCP = antimicrobial cleaning product; BCOP = bovine corneal opacity and permeability; BRD = background review document; EPA = U.S. Environmental Protection Agency.

[1] EPA classification system (EPA 2003a).

[2] Not Labeled = Category IV.

Table 6-9 Accuracy of the BCOP Test Method in Distinguishing Category IV Ocular Irritants from All Other Irritant Classes, as Defined by the EPA Classification System,[1] by Study and Overall

Data Source	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
Gautheron et al. (1994)	48	83	40/48	89	31/35	69	9/13	31	4/13	11	4/35
Balls et al. (1995)	54	93	50/54	92	48/52	100	2/2	0	0/2	8	4/52
Swanson et al. (1995)	10	90	9/10	100	9/9	0	0/1	100	1/1	0	0/9
Southee (1998)	15	93	14/15	93	13/14	100	1/1	0	0/1	7	0/14
Swanson and Harbell (2000)	8	75	6/8	100	6/6	0	0/2	100	2/2	0	0/6
Bailey et al. (2004)	13	62	8/13	75	3/4	44	4/9	56	5/9	25	1/4
AMCP BRD (2008)	66	79	52/66	98	47/48	28	5/18	72	13/18	2	1/48
Overall	187	83	155/187	94	134/142	47	21/45	53	24/45	6	8/142

Abbreviations: AMCP = antimicrobial cleaning products; BCOP = bovine corneal opacity and permeability; BRD = background review document; EPA = U.S. Environmental Protection Agency; N = number of substances included in this analysis; No. = data used to calculate the percentage.

[1] EPA classification system (EPA 2003a): Category IV vs. Categories I/II/III.

Among the eight false negatives for the EPA system, 100% (8/8) were EPA Category III substances based on Draize test data. For 38% (3/8) of these substances, the categorization was based on at least one rabbit with a corneal opacity score of 1 that was not resolved until Day 3 of the study. Another substance was categorized based on all six rabbits with a conjunctival redness score of 3 that was not resolved until Day 7 of the study. Among the seven false negative substances for which chemical class and/or physical properties could be assigned, 71% (5/7) were from discordant classes that have previously been identified for the BCOP test method (i.e., either ketones or solids; see also ICCVAM 2006a). Chemical class information was unavailable for the one substance that was from the AMCP BRD 2008 (**Table 6-10**).

Table 6-10 BCOP False Negative Substances[1]

Substance (Discordant Class Y/N)	*In Vivo* Classification					*In Vivo* Scores		
	EPA	GHS	EU	FHSA-20%	FHSA-67%	N	Corneal Opacity: Score (Day Cleared)	Conjunctival Redness: Score (Day Cleared)
Dimethylbiquanide (Y)	III	NC	NL	Irr	Irr	3	N=1 1(2) N=1 1(3)	N=2 2(3)
EDTA (Y)	III	NC	NL	Irr	Irr	3	N=1 1(3)	N=3 2(2)
Iminodibenzyl (Y)	III	NC	NL	Irr	Irr	3	N=3 1(2)	-
Magnesium Carbonate (Y)	III	NC	NL	Irr	Irr	3	N=1 1(2) N=1 1(3)	-
Methylcyclopentane (Y)	III	NC	NL	NL	NL	6	-	N=1 2(3)
Polyalkenylsuccinate ester/amine salt (N)	III	SCNM	SCNM	Irr	Irr	6	N=2 1(2)	N=1 2(6), N=3 2(2) N=1 3(2) N=1 3(6)
Tween 20 (N)	III	NC	NL	Irr	FTR	4	-	N=2 2(2)
Compound I (Disinfectant/ Cleaner; Unknown)	III	SCNM	SCNM	NI	NI	6	N=1 1(2)	N=2 1(2)
L-Aspartic acid (Y)	SCNM	SCNM	SCNM	Irr	Irr	3	N=1 1(3), N=1 1(>3) N=1 3(>3)	N=3 3(2)
DL-Glutamic acid (Y)	SCNM	SCNM	SCNM	Irr	FTR	3	N=1 1(2)	-

Abbreviations: BCOP = bovine corneal opacity and permeability; EPA = U.S. Environmental Protection Agency; EU = European Union; FTR = further testing required; GHS = Globally Harmonized System; Irr = irritant; N = number of animals; NC = Not Classified (as irritant); NL = Not Labeled (as irritant); SCNM = study criteria not met.

For the purposes of this evaluation, *clearing* is defined in the EPA hazard classification system as corneal opacity or iritis scores = 0 or redness or chemosis scores = 1; in the GHS and EU hazard classification systems as corneal opacity, iritis, redness, or chemosis scores = 0.

[1] False negative compounds (shaded here) are those that test as nonirritants *in vitro* but are mild, moderate, or severe ocular irritants/corrosive *in vivo*, i.e., EPA Categories I, II, and III; GHS Categories 1, 2A, and 2B; and EU R41 and R36.

6.3.6 Discordant Results According to the EPA Classification System

In order to evaluate discordant responses of the BCOP test method relative to the *in vivo* hazard classification, several accuracy subanalyses were performed. These included specific classes of chemicals with sufficiently robust numbers of substances (n ≥5), as well as certain properties of interest considered relevant to ocular toxicity testing (e.g., pesticides, surfactants, pH, physical form). **Table 6-11** shows some notable trends in the performance of the BCOP test method among these subgroups of substances. According to the EPA classification system, alcohols are the chemical class most consistently overpredicted by the BCOP test method. Nine of the 41 overpredicted substances were alcohols. Additional chemical classes represented among the overpredicted substances were hydrocarbons (6), carboxylic acids (5), ketones (4), esters (4), ethers (3), inorganic (1), and onium compounds (1). Among the substances labeled as surfactants, the BCOP test method overpredicted 32% (7/22).

Thirty-seven of the substances overpredicted by the BCOP test method were liquids and four were solids. Considering the proportion of the total available database, liquids (89/121 or 74%) appear more likely than solids (32/121 or 26%) to be overpredicted by the BCOP test method. Among the 22 substances labeled as surfactants, the BCOP test method overpredicted 32% (7/22).

According to the EPA classification system (see **Annex III**), the BCOP test method underpredicted relatively few substances (i.e., false negatives). Alcohols (2), esters (2), and heterocyclic compounds were most often underpredicted. As can be seen in **Table 6-11**, the 19 irritant substances labeled as surfactants were rarely underpredicted by the BCOP test method (9% [1/11] Category 1 substances were underpredicted; no Category II were underpredicted and 17% [1/6] Category III substances were underpredicted).

Nine of the substances underpredicted by the BCOP test method were solids, and nine were liquids. Given the proportion of the total available database, the BCOP test method appears more likely to underpredict solids (32/121 or 26%) than liquids (89/121 or 74%).

Table 6-12 shows the effects on the BCOP test method performance statistics of excluding from the data set problematic classes (i.e., those that gave the most discordant results according to the EPA classification system) identified in the BCOP BRD (ICCVAM 2006a). In general, the exclusion of alcohols, ketones, or solids individually resulted in small changes in the performance statistics. Exclusion of both alcohols and ketones improved the overall classification rate: 56% (54/96) versus 51% (62/121) for all compounds in the database. The classification of ocular corrosives/severe irritants was most improved by the exclusion of problematic classes. Using the entire database, 75% (24/32) of severe ocular corrosives/severe irritants were accurately classified. Removal of solids resulted in 86% (18/21) correct classification. Removal of alcohols, ketones, and solids resulted in correct classification of 90% (18/20) of Category I substances.

Table 6-11 Under- and Overprediction of the BCOP Test Method Using the EPA Classification System[1] in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method by Chemical Class or Physical Property

Category	N	Underprediction (*In Vivo*/*In Vitro*)						Overprediction (*In Vivo*/*In Vitro*)					
		Severe (Category I)			Moderate (Category II)		Mild (Cat III)	Moderate (Cat II)	Mild (Category III)		Not Labeled (Category IV)		
		II	III	IV	III	IV	IV	I	I	II	I	II	III
Overall	121	13% (4/32)	13% (4/32)	0% (0/32)	18% (3/17)	0% (0/17)	16% (7/45)	47% (8/17)	29% (13/45)	20% (9/45)	4% (1/27)	0% (0/27)	37% (10/27)
Chemical Class[2]													
Alcohol	17	50% (1/2)	50% (1/2)	0% (0/2)	0% (0/6)	0% (0/6)	0% (0/5)	67% (4/6)	80% (4/5)	20% (1/5)	0% (0/4)	0% (0/4)	0% (0/4)
Amine\Amidine	7	0% (0/2)	0% (0/2)	0% (0/2)	0/0	0/0	50% (2/4)	0/0	0% (0/4)	25% (1/4)	0% (0/1)	0% (0/1)	0% (0/1)
Carboxylic Acid	15	0% (0/7)	0% (0/7)	0% (0/7)	0% (0/2)	0% (0/2)	20% (1/5)	50% (1/2)	20% (1/5)	40% (2/5)	100% (1/1)	0% (0/1)	0% (0/1)
Ester	10	0% (0/1)	0% (0/1)	0% (0/1)	25% (1/4)	0% (0/4)	20% (1/5)	50% (2/4)	0% (0/5)	40% (2/5)	0/0	0/0	0/0
Ether	6	0/0	0/0	0/0	0% (0/4)	0% (0/4)	0% (0/2)	100% (1/1)	67% (2/3)	0% (0/3)	0% (0/4)	0% (0/4)	0% (0/4)
Heterocyclic	12	0% (0/5)	20% (1/5)	0% (0/5)	0/0	0/0	25% (1/4)	0% (0/1)	20% (1/5)	0% (0/5)	0% (0/1)	0% (0/1)	0% (0/1)
Hydrocarbon	11	0/0	0/0	0/0	0% (0/4)	0% (0/4)	0% (0/2)	0/0	20% (1/5)	40% (2/5)	0% (0/6)	0% (0/6)	50% (3/6)
Inorganics	7	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/1)	0% (0/1)	33% (1/3)	100% (1/1)	0% (0/3)	0% (0/3)	0/0	0/0	0/0
Ketone	10	0/0	0/0	0/0	0% (0/1)	0% (0/1)	14% (1/7)	100% (1/1)	43% (3/7)	0% (0/7)	0% (0/1)	0% (0/1)	0% (0/1)

Category	N	Underprediction (*In Vivo*/*In Vitro*)						Overprediction (*In Vivo*/*In Vitro*)					
		Severe (Category I)			Moderate (Category II)		Mild (Cat III)	Moderate (Cat II)	Mild (Category III)		Not Labeled (Category IV)		
		II	III	IV	III	IV	IV	I	I	II	I	II	III
Onium Compound	10	17% (1/6)	0% (0/6)	0% (0/6)	0% (0/1)	0% (0/1)	0% (0/2)	100% (1/1)	0% (0/2)	0% (0/2)	0% (0/1)	0% (0/1)	0% (0/1)
Polyether	2	0/0	0/0	0/0	0/0	0/0	100% (1/1)	0/0	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/1)
Properties of Interest													
Liquids	89	10% (2/21)	5% (1/21)	0% (0/21)	20% (3/15)	0% (0/15)	9% (3/33)	47% (7/15)	36% (12/33)	27% (9/33)	0% (0/20)	0% (0/20)	45 (9/20)
Solids	32	18% (2/11)	27% (3/11)	0% (0/11)	0% (0/2)	0% (0/2)	36% (4/11)	50% (1/2)	9% (1/11)	0% (0/11)	14% (1/7)	0% (0/7)	14% (1/7)
Pesticide	9	20% (1/5)	20% (1/5)	0% (0/5)	0/0	0/0	0% (0/4)	0/0	67% (2/3)	0% (0/3)	0/0	0/0	0/0
Surfactant–Total	22	0% (0/11)	9% (1/11)	0% (0/11)	0% (0/2)	0% (0/2)	17% (1/6)	100% (2/2)	33% (2/6)	33% (2/6)	0% (0/3)	0% (0/3)	33% (1/3)
-nonionic	11	0% (0/4)	0% (0/4)	0% (0/4)	0% (0/1)	0% (0/1)	33% (1/3)	100% (1/1)	67% (2/3)	0% (0/3)	0% (0/3)	0% (0/3)	33% (1/3)
-anionic	8	0% (0/5)	20% (1/5)	0% (0/5)	0/0	0/0	0% (0/2)	0/0	0% (0/2)	100% (2/2)	0% (0/1)	0% (0/1)	100% (1/1)
-cationic	6	0% (0/4)	0% (0/4)	0% (0/4)	0% (0/1)	0% (0/1)	0% (0/1)	100% (1/1)	0% (0/1)	0% (0/1)	0/0	0/0	0/0

Abbreviations: BCOP = bovine corneal opacity and permeability; EPA = U.S. Environmental Protection Agency.

[1] EPA classification system (EPA 2003a).

[2] Chemical classes included in this table are represented by at least five substances tested in the BCOP test method and assignments are based upon National Library of Medicine medical subject heading (MeSH) categories (www.nlm.nih.gov/mesh) as defined in **Annex I**.

As shown in **Table 6-11**, hydrocarbons were also noted as discordant when the BCOP test method was evaluated for its ability to identify all hazard categories. Among the 11 hydrocarbons in the validation database, the BCOP test method overpredicted 55% (6/11) (**Table 6-11**). Compared to the entire database, exclusion of hydrocarbons resulted in only modest improvement in overall correct classification (52% [57/110] versus 51% [62/121]) and identification of Category IV substances (62% [13/21] versus 59% [16/27]) (**Table 6-12**). Accuracy increased from 85% (103/121) to 86% (95/110), and the false positive rate decreased from 41% (11/27) to 38% (8/21). However, exclusion of hydrocarbons slightly increased the false negative rate from 7% (7/94) to 8% (7/89).

Table 6-13 shows how the ability of the BCOP test method to distinguish Category IV substances was affected by exclusion of problematic classes from the data set. Exclusion of problematic classes individually or in combination had little effect on accuracy (85% versus 82% to 87%), sensitivity (91% to 96%), or specificity (44% to 63%). The overall false positive rate of 7% (7/94) showed the largest decrease following the exclusion of solids, the false positive rate dropping to 4% (3/69).

6.4 EU Classification System: BCOP Test Method Accuracy

The six reports used in the accuracy evaluation (Gautheron et al. 1994, Balls et al. 1995, Swanson et al. 1995, Southee 1998, Swanson and Harbell 2000, and Bailey et al. 2004) included BCOP data on 118 substances that had sufficient *in vivo* data to be assigned an ocular irritancy classification according to the EU classification system (EU 2004) (see **Annex III**). Among these studies, Gautheron et al. (1994), Balls et al. (1995), and Southee (1998) provided BCOP data for substances tested in multiple laboratories and thus required that a consensus *in vitro* classification be assigned to each substance. Based on results from *in vivo* rabbit eye experiments, 28% (33/118) were classified as R41, 14% (21/118) were classified as R36, and 54% (64/118) were classified as Not Labeled.

6.4.1 Identification of R41 Substances (Ocular Corrosives/Severe Irritants)

The BCOP test method correctly identified 79% (26/33) and 73% (24/33) of the R41 substances using decision criteria that defined *in vitro* scores ≥55.1 as R41 and *in vitro* scores ≥75 as R41, respectively (**Table 6-2**). Using decision criteria that defined *in vitro* scores ≥55.1 as R41, all seven substances that were underpredicted by the BCOP test method were classified as R36. Using decision criteria that defined *in vitro* scores ≥75 as R41, all nine substances that were underpredicted by the BCOP test method were classified as R36.

6.4.2 Identification of R36 Substances (Irritants)

For the 21 substances that could be evaluated, the BCOP test method correctly identified 52% (11/21) as R36, while 48% (10/21) were overpredicted using decision criteria defining *in vitro* scores ≥55.1 as R41 (**Table 6-14**). Using decision criteria that defined *in vitro* scores ≥75 as R41, the BCOP test method correctly identified 67% (14/21) as R36, while 29% (6/21) were overpredicted and 4% (1/21) were underpredicted (**Table 6-2**).

Table 6-12 Performance of the BCOP Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the EPA Classification System,[1] with Discordant Chemical and Physical Classes Excluded

BCOP	Overall Correct Classification	Severe (Category I)		Moderate (Category II)			Mild (Category III)			Not Labeled (Category IV)	
		Actual	Under	Over	Actual	Under	Over	Actual	Under	Over	Actual
Overall	51% (62/121)	75% (24/32)	25% (8/32)	47% (8/17)	35% (6/17)	18% (3/17)	49% (22/45)	36% (16/45)	15% (7/45)	41% (11/27)	59% (16/27)
Without Alcohols	54% (57/105)	73% (24/33)	43% (14/33)	36% (4/11)	36% (4/11)	27% (3/11)	43% (17/40)	40% (16/40)	18% (7/40)	46% (11/24)	54% (13/24)
Without Ketones	53% (59/112)	75% (24/32)	25% (8/32)	44% (7/16)	38% (6/16)	19% (3/16)	47% (18/38)	37% (14/38)	16% (6/38)	42% (11/26)	58% (15/26)
Without Solids	48% (43/89)	86% (18/21)	14% (3/21)	47% (7/15)	33% (5/15)	20% (3/15)	64% (21/33)	27% (9/33)	9% (3/33)	45% (9/20)	55% (11/20)
Without Alcohols and Ketones	56% (54/96)	80% (24/30)	20% (6/30)	30% (3/10)	40% (4/10)	30% (3/10)	39% (13/33)	42% (14/33)	18% (6/33)	48% (11/23)	52% (12/23)
Without Alcohols, Ketones, and Solids	54% (35/65)	90% (18/20)	10% (2/20)	25% (2/8)	38% (3/8)	37% (3/8)	57% (12/21)	33% (7/21)	10% (2/21)	56% (9/16)	44% (7/16)
Without Hydrocarbons	52% (57/110)	75% (24/32)	25% (8/32)	47% (8/17)	35% (6/17)	18% (3/17)	48% (19/40)	35% (14/40)	17% (7/40)	38% (8/21)	62% (13/21)

Abbreviations: BCOP = bovine corneal opacity and permeability; EPA = U.S. Environmental Protection Agency.

[1] EPA classification system (EPA 2003a).

Table 6-13 Accuracy of the BCOP Test Method in Distinguishing Category IV Ocular Irritants from All Other Irritant Classes, as Defined by the EPA Classification System,[1] with Discordant Chemical and Physical Classes Excluded

BCOP	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
Overall	187	83	155/187	94	134/142	47	21/45	53	24/45	6	8/142
Without Alcohols	105	83	87/105	91	74/81	63	13/24	46	11/24	9	7/81
Without Ketones	112	85	95/112	93	80/86	58	15/26	42	11/26	7	6/86
Without Solids	89	87	77/89	96	66/69	55	11/20	45	9/20	4	3/69
Without Alcohols and Ketones	96	82	79/96	92	67/73	52	12/23	48	11/23	8	6/73
Without Alcohols, Ketones, and Solids	65	82	53/65	96	47/49	44	7/16	56	9/16	4	2/49
Without Hydrocarbons	110	86	95/110	92	82/89	62	13/21	38	8/21	8	7/89

Abbreviations: BCOP = bovine corneal opacity and permeability; EPA = U.S. Environmental Protection Agency;.N = number of substances included in this analysis/total number of substances in the study; No. = data used to calculate the percentage.

[1] EPA classification system (EPA 2003a); Category IV vs. Categories I/II/III.

6.4.3 Identification of Not Labeled Substances

Regardless of the decision criteria used to define R41, for the 64 substances that could be evaluated, the BCOP test method correctly identified 34% (22/64) as Not Labeled, while 66% (42/64) were overpredicted (**Table 6-14**).

6.4.4 Ability to Distinguish Not Labeled Substances from All Other Classes

In addition to evaluating the ability of the BCOP test method to identify each individual ocular hazard category according to the EU classification system, ICCVAM also evaluated the ability of the BCOP test method to distinguish Not Labeled substances from all other irritant classes. Using this approach for the 118 substances considered, the BCOP test method has an accuracy of 64% (76/118), a sensitivity of 100% (54/54), a specificity of 34% (22/64), a false positive rate of 66% (42/64), and a false negative rate of 0% (0/54) (**Table 6-15**).

Table 6-14 Performance of the BCOP Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the EU Classification System,[1] by Study and Overall

Data Source	Overall Correct Classification	Severe (R41)		Moderate (R36)			Mild			Not Labeled[2]	
		Actual	Under	Over	Actual	Under	Over	Actual	Under	Over	Actual
Gautheron et al. (1994)	42% (18/43)	75% (6/8)	25% (2/8)	50% (2/4)	50% (2/4)	0% (0/4)	NA	NA	NA	68% (21/31)	32% (10/31)
Balls et al. (1995)	54% (27/50)	74% (14/19)	26% (5/19)	47% (7/15)	53% (8/15)	0% (0/15)	NA	NA	NA	69% (11/16)	31% (5/16)
Swanson et al. (1995)	50% (6/12)	100% (6/6)	0% (0/6)	0% (0/0)	0% (0/0)	0% (0/0)	NA	NA	NA	100% (6/6)	0% (0/6)
Southee (1998)	60% (9/15)	67% (4/6)	33% (2/6)	40% (2/5)	60% (3/5)	0% (0/5)	NA	NA	NA	50% (2/4)	50% (2/4)
Swanson and Harbell (2000)	38% (3/8)	100% (1/1)	0% (0/1)	50% (2/4)	50% (2/4)	0% (0/4)	NA	NA	NA	100% (3/3)	0% (0/3)
Bailey et al. (2004)	46% (6/13)	67% (2/3)	33% (1/3)	0% (0/0)	0% (0/0)	0% (0/0)	NA	NA	NA	60% (6/10)	40% (4/10)
AMCP BRD (2008)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Overall	50% (59/118)	79% (26/33)	21% (7/33)	48% (10/21)	52% (11/21)	0% (0/21)	NA	NA	NA	66% (42/64)	34% (22/64)

Abbreviations: AMCP = antimicrobial cleaning product; BCOP = bovine corneal opacity and permeability; BRD = background review document; EU = European Union; NA = not applicable.

[1] EU classification system (EU 2001).

[2] Not Labeled = Not Labeled as Irritant.

Table 6-15 Accuracy of the BCOP Test Method in Distinguishing Not Labeled Substances from All Other Irritant Classes, as Defined by the EU Classification System,[1] by Study and Overall

Data Source	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
Gautheron et al. (1994)	43	51	22/43	100	12/12	32	10/31	68	21/31	0	0/12
Balls et al. (1995)	50	78	39/50	100	34/34	31	5/16	69	11/16	0	0/34
Swanson et al. (1995)	12	50	6/12	100	6/6	0	0/6	100	6/6	0	0/6
Southee (1998)	15	87	13/15	100	11/11	50	2/4	50	2/4	0	0/11
Swanson and Harbell (2000)	8	63	5/8	100	5/5	0	0/3	100	3/3	0	0/5
Bailey et al. (2004)	13	54	7/13	100	3/3	40	4/10	60	6/10	0	0/3
AMCP BRD (2008)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Overall	118	64	76/118	100	54/54	34	22/64	66	42/64	0	0/54

Abbreviations: BCOP = bovine corneal opacity and permeability; BRD = background review document; EU = European Union; N = number of substances included in this analysis; No. = data used to calculate the percentage.

[1] EU classification system (EU 2001): Not Labeled vs. R41/R36.

As detailed below, the results from each individual study were also evaluated separately.

Gautheron et al. (1994): Based upon the *in vivo* rabbit data, 43 substances could be assigned EU classifications. Based on these 43 substances, the BCOP test method has an accuracy of 51% (22/43), sensitivity of 100% (12/12), specificity of 32% (10/31), false positive rate of 68% (21/31), and a false negative rate of 0% (0/12) (**Table 6-15**).

Balls et al. (1995): Based upon the *in vivo* rabbit data, 50 substances could be assigned EU classifications. Based on these 50 substances, the BCOP test method has an accuracy of 78% (39/50), sensitivity of 100% (34/34), specificity of 31% (5/16), false positive rate of 69% (11/16), and a false negative rate of 0% (0/34) (**Table 6-15**).

Swanson et al. (1995): Based upon the *in vivo* rabbit data, 12 substances could be assigned EU classifications. Based on these 12 substances, the BCOP test method has an accuracy of 50% (6/12), sensitivity of 100% (6/6), specificity of 0% (0/6), false positive rate of 100% (6/6), and a false negative rate of 0% (0/6) (**Table 6-15**).

Southee (1998): Based upon the *in vivo* rabbit data, 15 substances could be assigned EU classifications. Based on these 15 substances, the BCOP test method has an accuracy of 87% (13/15), sensitivity of 100% (11/11), specificity of 50% (2/4), false positive rate of 50% (2/4), and a false negative rate of 0% (0/11) (**Table 6-15**).

Swanson and Harbell (2000): Based upon the *in vivo* rabbit data, eight substances could be assigned EU classifications. Based on these eight substances, the BCOP test method has an accuracy of 63% (5/8), sensitivity of 100% (5/5), specificity of 0% (0/3), false positive rate of 100% (3/3), and a false negative rate of 0% (0/5) (**Table 6-15**).

Bailey et al. (2004): Based upon the *in vivo* rabbit data, 13 substances could be assigned EU classifications. Based on these 13 substances, the BCOP test method has an accuracy of 54% (7/13), sensitivity of 100% (3/3), specificity of 40% (4/10), false positive rate of 60% (6/10), and a false negative rate of 0% (0/3) (**Table 6-15**).

6.4.5 Discordant Results According to the EU Classification System

In order to evaluate discordant responses of the BCOP test method relative to the *in vivo* hazard classification, several accuracy subanalyses were performed. These included specific classes of chemicals with sufficiently robust numbers of substances (n ≥5), as well as certain properties of interest considered relevant to ocular toxicity testing (e.g., surfactants and physical form, respectively).

Table 6-16 shows some notable trends in the performance of the BCOP test method among these subgroups of substances. The chemical class of substances that was most consistently overpredicted according to the EU classification system by the BCOP test method was hydrocarbons. Seven of the 42 overpredicted substances were hydrocarbons. Additional chemical classes represented among the overpredicted substances were ketones (5), esters (5), carboxylic acids (4), alcohols (3), and heterocyclic compounds (3). Among the 24 substances labeled as surfactants, the BCOP test method overpredicted 25% (6/24).

The BCOP test method overpredicted 35 liquids and 7 solids. Considering the proportion of the total available database, the BCOP test method appears more likely to overpredict liquids (88/118 or 75%) than solids (30/118 or 25%).

According to the EU classification system (see **Annex III**), alcohols (2) were most often underpredicted (i.e., false negatives) by the BCOP test method. As can be seen in **Table 6-16**, none of the 24 substances labeled as surfactants was underpredicted by the BCOP test method (0% [0/24]).

The BCOP test method underpredicted five solids and one liquid. As a proportion of the total available database, solids (30/118 or 25%) appear more likely than liquids (88/118 or 75%) to be underpredicted by the BCOP test method.

Table 6-17 shows how the BCOP test method performance statistics were affected by excluding from the data set problematic classes (i.e., those that gave the most discordant results, according to the EU classification system) identified in the BCOP BRD (ICCVAM 2006a). In general, the exclusion of alcohols, ketones, or solids individually resulted in small changes in the performance statistics. Exclusion of both alcohols and ketones improved the overall classification rate: 53% (50/94) versus 50% (59/118) for all compounds in the database. The classification of ocular corrosives/severe irritants was most improved by the exclusion of problematic classes. Using the entire database, 79% (26/33) of severe ocular corrosives/severe irritants were accurately classified, while removal of solids resulted in 91% (21/23) correct classification. Removal of alcohols, ketones, and solids resulted in correct classification of 95% (20/21) ocular corrosives/severe irritants. Evaluation of overpredicted substances shows 64% (7/11) of hydrocarbons were overpredicted (**Table 6-16**). Compared to the entire database, exclusion of hydrocarbons improved overall correct classification (52% [56/107)] versus 50% [62/121]) and slightly improved identification of substances Not Labeled as Irritants (36% [19/53] versus 34% [22/64]) (**Table 6-17**).

Table 6-16 Under- and Overprediction of the BCOP Test Method Using the EU Classification System[1] in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method by Chemical Class or Physical Property

Category	N	Underprediction (*In Vivo/In Vitro*)			Overprediction (*In Vivo/In Vitro*)		
		Severe (R41)		Moderate (R36)	Moderate (R36)	Not Labeled (NL)[2]	
		R36	NL	NL	R41	R41	R36
Overall	118	21% (7/33)	0% (0/33)	0% (0/21)	48% (10/21)	13% (8/64)	38% (24/64)
Chemical Class[3]							
Alcohol	16	67% (2/3)	0% (0/3)	0% (0/6)	50% (3/6)	0% (0/7)	0% (0/7)
Amine/Amidine	6	0% (0/2)	0% (0/2)	0/0	0/0	0% (0/4)	25% (1/4)
Carboxylic acid	13	25% (1/4)	0% (0/4)	0% (0/3)	33% (1/3)	33% (2/6)	17% (1/6)
Ester	10	0% (0/2)	0% (0/2)	0% (0/3)	33% (1/3)	40% (2/5)	40% (2/5)
Ether	6	0% (0/1)	0% (0/1)	0% (0/1)	100% (1/1)	0% (0/2)	0% (0/2)
Heterocyclic	13	17% (1/6)	0% (0/6)	0% (0/1)	0% (0/1)	0% (0/6)	50% (3/6)
Hydrocarbon	11	0/0	0/0	0/0	0/0	18% (2/11)	45% (5/11)
Inorganics	7	0% (0/5)	0% (0/5)	0% (0/1)	0% (0/1)	0% (0/2)	50% (1/2)
Ketone	9	0/0	0/0	0% (0/2)	100% (2/2)	14% (1/7)	28% (2/7)
Onium compound	11	13% (1/8)	0% (0/8)	0% (0/1)	0% (0/1)	0% (0/2)	50% (1/2)
Polyether	2	0/0	0/0	0/0	0/0	0% (0/2)	0% (0/2)
Properties of Interest							
Liquids	88	4% (1/23)	0% (0/23)	0% (0/18)	50% (9/18)	17% (8/47)	38% (18/47)
Solids	30	50% (5/10)	0% (0/10)	0% (0/2)	50% (1/2)	0% (0/17)	35% (6/17)
Pesticide	7	50% (2/4)	0% (0/4)	0% (0/1)	100% (1/1)	0% (0/2)	50% (1/2)
Surfactants: total	24	0% (0/13)	0% (0/13)	0% (0/2)	50% (1/2)	22% (2/9)	33% (3/9)
Surfactants: nonionic	11	0% (0/5)	0% (0/5)	0% (0/1)	100% (1/1)	0% (0/5)	20% (1/5)

Category	N	Underprediction (*In Vivo/In Vitro*)			Overprediction (*In Vivo/In Vitro*)		
		Severe (R41)		Moderate (R36)	Moderate (R36)	Not Labeled (NL)[2]	
		R36	NL	NL	R41	R41	R36
Surfactants: anionic	9	0% (0/4)	0% (0/4)	0% (0/1)	0% (0/1)	50% (2/4)	50% (2/4)
Surfactants: cationic	7	0% (0/6)	0% (0/6)	0/0	0/0	0% (0/1)	100% (1/1)

Abbreviations: BCOP= bovine corneal opacity and permeability; EU = European Union; N = number of substances used in this analysis/total number of substances in the study.

[1] EU classification system (EU 2001).

[2] Not Labeled (NL) = Not Labeled as Irritant.

[3] Chemical classes included in this table are represented by at least five substances tested in the BCOP test method, and assignments are based upon National Library of Medicine medical subject heading (MeSH) categories (www.nlm.nih.gov/mesh) as defined in Annex I.

Table 6-18 shows how the ability of the BCOP test method to distinguish substances not labeled as irritants was affected by exclusion of problematic classes from the data set. Exclusion of problematic classes individually or in combination had a minimal effect on accuracy (64% versus 60% to 66%) and specificity (24% to 35%). Sensitivity was 100% using the overall database and therefore remained unchanged. None of the R41 substances was classified by the BCOP test method as not labeled as an irritant. Exclusion of hydrocarbons resulted in modest improvement in overall performance in identifying substances not labeled as irritants (see **Table 6-18**). Accuracy increased from 64% (76/118) to 68% (73/107). The false positive rate decreased from 66% (42/64) to 64% (34/53), while the false negative rate remained 0% (0/54 versus 0/54).

Table 6-17 Performance of the BCOP Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the EU Classification System,[1] with Discordant Chemical and Physical Classes Excluded

BCOP	Overall Correct Classification	Severe (R41)		Moderate (R36)			Mild			Not Labeled[2]	
		Actual	Under	Over	Actual	Under	Over	Actual	Under	Over	Actual
Overall	50% (59/118)	79% (26/33)	21% (7/33)	48% (10/21)	52% (11/21)	0% (0/21)	NA	NA	NA	66% (42/64)	34% (22/64)
Without Alcohols	50% (52/103)	83% (25/30)	17% (5/30)	47% (7/15)	53% (8/15)	0% (0/15)	NA	NA	NA	67% (39/58)	33% (19/58)
Without Ketones	52% (59/109)	79% (26/33)	21% (7/33)	42% (8/19)	58% (11/19)	0% (0/19)	NA	NA	NA	65% (37/57)	35% (20/57)
Without Solids	49% (43/88)	91% (21/23)	9% (2/23)	50% (9/18)	50% (9/18)	0% (0/18)	NA	NA	NA	72% (34/47)	28% (13/47)
Without Alcohols and Ketones	53% (50/94)	83% (25/30)	17% (5/30)	38% (5/13)	62% (8/13)	0% (0/13)	NA	NA	NA	67% (34/51)	33% (17/51)
Without Alcohols, Ketones, and Solids	52% (34/65)	95% (20/21)	5% (1/21)	40% (4/10)	60% (6/10)	0% (0/10)	NA	NA	NA	76% (26/34)	24% (8/34)
Without Hydrocarbons	52% (56/107)	79% (26/33)	21% (7/33)	48% (10/21)	52% (11/21)	0% (0/21)	NA	NA	NA	64% (34/53)	36% (19/53)

Abbreviations: BCOP = bovine corneal opacity and permeability; EU = European Union; NA = not applicable.

[1] EU classification system (EU 2001).

[2] Not Labeled = Not Labeled as Irritant.

Table 6-18 Accuracy of the BCOP Test Method in Distinguishing Substances Not Labeled as Irritants from All Other Irritant Classes, as Defined by the EU Classification System,[1] with Discordant Chemical and Physical Classes Excluded

BCOP	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
Overall	118	64	76/118	100	54/54	34	22/64	66	42/64	0	0/54
Without Alcohols	103	62	64/103	100	45/45	33	19/58	67	39/58	0	0/45
Without Ketones	109	66	72/109	100	52/52	35	20/57	65	37/57	0	0/52
Without Solids	88	61	54/88	100	41/41	28	13/47	72	34/47	0	0/41
Without Alcohols and Ketones	94	64	60/94	100	43/43	33	17/51	67	34/51	0	0/43
Without Alcohols, Ketones, and Solids	65	60	39/65	100	31/31	24	8/34	76	26/34	0	0/31
Without Hydrocarbons	107	68	73/107	100	54/54	36	19/53	64	34/53	0	0/54

Abbreviations: BCOP = bovine corneal opacity and permeability; EU = European Union; N = number of substances included in this analysis/the total number of substances in the study; No. = data used to calculate the percentage.

[1] EU classification system (EU 2001): Not Labeled vs. R41/R36.

6.5 FHSA Classification System: BCOP Test Method Accuracy

The six reports used in the accuracy evaluation (Gautheron et al. 1994, Balls et al. 1995, Swanson et al. 1995, Southee 1998, Swanson and Harbell 2000, and Bailey et al. 2004) included BCOP data on 194 and 179 substances that had sufficient *in vivo* data to be assigned an ocular irritancy classification according to the FHSA-20% and FHSA-67% classification systems, respectively (FHSA 2005) (see **Annex III**). Among these studies, Gautheron et al. (1994), Balls et al. (1995), and Southee (1998) provided BCOP data for substances tested in multiple laboratories and thus required that a consensus *in vitro* classification be assigned to each substance. Based on results from *in vivo* rabbit eye experiments, 76% (147/194) and 74% (132/179) were classified as irritants in FHSA-20% and FHSA-67%, respectively, while 24% (47/194) and 26% (47/179) were classified as Not Labeled in FHSA-20% and FHSA-67%, respectively.

6.5.1 Ability to Distinguish Not Labeled Substances from All Other Classes

ICCVAM also evaluated the ability of the BCOP test method to distinguish Not Labeled substances from irritants using the FHSA-20% and FHSA-67% classification systems.

Ability to Distinguish Not Labeled Substances from All Other Classes using the FHSA-20% Classification System

ICCVAM evaluated the ability of the BCOP test method to distinguish Not Labeled substances from irritants using the FHSA-20% classification system. Using this approach for the 194 substances, the BCOP test method has an overall accuracy of 83% (161/194), a sensitivity of 95% (139/147), a specificity of 47% (22/47), a false positive rate of 53% (25/47), and a false negative rate of 5% (8/147) (**Table 6-19**).

As detailed below, the results from each individual study were also evaluated separately.

Gautheron et al. (1994): Based upon the *in vivo* rabbit data, 52 substances could be assigned an FHSA-20% classification. Based on these 52 substances, the BCOP test method has an accuracy of 83% (43/52), sensitivity of 88% (35/40), specificity of 67% (8/12), false positive rate of 33% (4/12), and a false negative rate of 13% (5/40) (**Table 6-19**).

Balls et al. (1995): Based upon the *in vivo* rabbit data, 58 substances could be assigned an FHSA-20% classification. Based on these 58 substances, the BCOP test method has an accuracy of 91% (53/58), sensitivity of 93% (50/54), specificity of 75% (3/4), false positive rate of 25% (1/4), and a false negative rate of 7% (4/54) (**Table 6-19**).

Swanson et al. (1995): Based upon the *in vivo* rabbit data, 9 substances could be assigned an FHSA-20% classification. Based on these 9 substances, the BCOP test method has an accuracy of 89% (8/9), sensitivity of 100% (8/8), specificity of 0% (0/1), false positive rate of 100% (1/1), and a false negative rate of 0% (0/8) (**Table 6-19**).

Southee (1998): Based upon the *in vivo* rabbit data, 15 substances could be assigned an FHSA-20% classification. Based on these 15 substances, the BCOP test method has an accuracy of 93% (14/15), sensitivity of 93% (13/14), specificity of 100% (1/1), false positive rate of 0% (0/1), and a false negative rate of 7% (1/14) (**Table 6-19**).

Swanson and Harbell (2000): Based upon the *in vivo* rabbit data, 8 substances could be assigned an FHSA-20% classification. Based on these 8 substances, the BCOP test method has an accuracy of 75% (6/8), sensitivity of 100% (6/6), specificity of 0% (0/2), false positive rate of 100% (2/2), and a false negative rate of 0% (0/6) (**Table 6-19**).

Bailey et al. (2004): Based upon the *in vivo* rabbit data, 15 substances could be assigned an FHSA-20% classification. Based on these 15 substances, the BCOP test method has an accuracy of 73% (11/15),

sensitivity of 88% (7/8), specificity of 57% (4/7), false positive rate of 43% (3/7), and a false negative rate of 13% (1/8) (**Table 6-19**).

AMCP BRD (2008): Based upon the *in vivo* rabbit data, 63 substances could be assigned an FHSA-20% classification. Based on these 63 substances, the BCOP test method has an accuracy of 67% (42/63), sensitivity of 100% (42/42), specificity of 0% (0/21), false positive rate of 100% (21/21), and a false negative rate of 0% (0/42) (**Table 6-19**).

Table 6-19 Accuracy of the BCOP Test Method in Distinguishing Not Labeled Substances from All Other Irritant Classes, as Defined by the FHSA-20% Classification System,[1] by Study and Overall

Data Source	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
Gautheron et al. (1994)	52	83	43/52	88	35/40	67	8/12	33	4/12	13	5/40
Balls et al. (1995)	58	91	53/58	93	50/54	75	3/4	25	1/4	7	4/54
Swanson et al. (1995)	9	89	8/9	100	8/8	0	0/1	100	1/1	0	0/8
Southee (1998)	15	93	14/15	93	13/14	100	1/1	0	0/1	7	1/14
Swanson and Harbell (2000)	8	75	6/8	100	6/6	0	0/2	100	2/2	0	0/6
Bailey et al. (2004)	15	73	11/15	88	7/8	57	4/7	43	3/7	13	1/8
AMCP BRD (2008)	63	67	42/63	100	42/42	0	0/21	100	21/21	0	0/42
Overall	194	83	161/194	95	139/147	47	22/47	53	25/47	5	8/147

Abbreviations: BCOP = bovine corneal opacity and permeability; FHSA = Federal Hazardous Substances Act; N = number of substances included in this analysis; No. = data used to calculate the percentage.

[1] FHSA-20% classification system (2005): Not Labeled vs. Irritant.

Ability to Distinguish Not Labeled Substances from All Other Classes using the FHSA-67% Classification System

ICCVAM evaluated the ability of the BCOP test method to distinguish Not Labeled substances from irritants using the FHSA-67% classification system. Using this approach for the 179 substances, the BCOP test method has an overall accuracy of 83% (148/179), a sensitivity of 95% (126/132), a specificity of 47% (22/47), a false positive rate of 53% (25/47), and a false negative rate of 5% (6/132) (**Table 6-20**).

As detailed below, the results from each individual study were also evaluated separately.

Gautheron et al. (1994): Based upon the *in vivo* rabbit data, 48 substances could be assigned an FHSA-67% classification. Based on these 48 substances, the BCOP test method has an accuracy of 83% (40/48), sensitivity of 89% (32/36), specificity of 67% (8/12), false positive rate of 33% (4/12), and a false negative rate of 11% (4/36) (**Table 6-20**).

Balls et al. (1995): Based upon the *in vivo* rabbit data, 52 substances could be assigned an FHSA-67% classification. Based on these 52 substances, the BCOP test method has an accuracy of 94% (49/52), sensitivity of 96% (46/48), specificity of 75% (3/4), false positive rate of 25% (1/4), and a false negative rate of 4% (2/48) (**Table 6-20**).

Swanson et al. (1995): Based upon the *in vivo* rabbit data, eight substances could be assigned an FHSA-67% classification. Based on these 8 substances, the BCOP test method has an accuracy of 88% (7/8), sensitivity of 100% (7/7), specificity of 0% (0/1), false positive rate of 100% (1/1), and a false negative rate of 0% (0/7) (**Table 6-20**).

Southee (1998): Based upon the *in vivo* rabbit data, 14 substances could be assigned an FHSA-67% classification. Based on these 14 substances, the BCOP test method has an accuracy of 100% (14/14), sensitivity of 100% (13/13), specificity of 100% (1/1), false positive rate of 0% (0/1), and a false negative rate of 0% (0/13) (**Table 6-20**).

Swanson and Harbell (2000): Based upon the *in vivo* rabbit data, seven substances could be assigned an FHSA-67% classification. Based on these 7 substances, the BCOP test method has an accuracy of 71% (5/7), sensitivity of 100% (5/5), specificity of 0% (0/2), false positive rate of 100% (2/2), and a false negative rate of 0% (0/5) (**Table 6-20**).

Bailey et al. (2004): Based upon the *in vivo* rabbit data, 14 substances could be assigned an FHSA-67% classification. Based on these 14 substances, the BCOP test method has an accuracy of 71% (10/14), sensitivity of 86% (6/7), specificity of 57% (4/7), false positive rate of 43% (3/7), and a false negative rate of 14% (1/7) (**Table 6-20**).

AMCP BRD (2008): Based upon the *in vivo* rabbit data, 63 substances could be assigned an FHSA-67% classification. Based on these 63 substances, the BCOP test method has an accuracy of 67% (42/63), sensitivity of 100% (42/42), specificity of 0% (0/21), false positive rate of 100% (21/21), and a false negative rate of 0% (0/42) (Table 6-20).

6.5.2 Discordant Results According to the FHSA Classification System

In order to evaluate discordant responses of the BCOP test method relative to the *in vivo* hazard classification, several accuracy subanalyses were performed. These included specific classes of chemicals with sufficiently robust numbers of substances (n ≥5), as well as certain properties of interest considered relevant to ocular toxicity testing (e.g., surfactants and physical form, respectively).

Discordant Results According to the FHSA-20% Classification System

Table 6-21 shows how the ability of the BCOP test method to distinguish substances not labeled as irritants was affected by exclusion of problematic classes from the data set. Exclusion of problematic classes individually or in combination had a minimal or no effect on accuracy (83% versus 80% to 84%), specificity (94% to 98%) and specificity (36% to 47%). Exclusion of hydrocarbons also resulted no significant improvement in overall performance in identifying substances not labeled as irritants. However, a slightly higher false positive rate and slightly lower false negative rate occurred with exclusion of discordant classes (see **Table 6-21**).

Discordant Results According to the FHSA-67% Classification System

Table 6-22 shows how the ability of the BCOP test method to distinguish substances not labeled as irritants was affected by exclusion of problematic classes from the data set. Exclusion of problematic classes individually or in combination had a minimal or no effect on accuracy (83% versus 80% to 83%), specificity (95% to 99%) and specificity (36% to 47%). Exclusion of hydrocarbons also resulted no significant improvement in overall performance in identifying substances not labeled as irritants. However, a slightly higher false positive rate and slightly lower false negative rate occurred with exclusion of discordant classes (see **Table 6-22**).

Table 6-20 Accuracy of the BCOP Test Method in Distinguishing Not Labeled Substances from All Other Irritant Classes, as Defined by the FHSA-67% Classification System,[1] by Study and Overall

Data Source	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
Gautheron et al. (1994)	48	83	40/48	89	32/36	67	8/12	33	4/12	11	4/36
Balls et al. (1995)	52	94	49/52	96	46/48	75	3/4	25	1/4	4	2/48
Swanson et al. (1995)	8	88	7/8	100	7/7	0	0/1	100	1/1	0	0/7
Southee (1998)	14	100	14/14	100	13/13	100	1/1	0	0/1	0	0/13
Swanson and Harbell (2000)	7	71	5/7	100	5/5	0	0/2	100	2/2	0	0/5
Bailey et al. (2004)	14	71	10/14	86	6/7	57	4/7	43	3/7	14	1/7
AMCP BRD (2008)	59	64	38/59	100	38/38	0	0/21	100	21/21	0	0/38
Overall	179	83	148/179	95	126/132	47	22/47	53	25/47	5	6/132

Abbreviations: BCOP = bovine corneal opacity and permeability; FHSA = Federal Hazardous Substances Act; N = number of substances included in this analysis; No. = data used to calculate the percentage.

[1] FHSA-67% classification system (2005): Not Labeled vs. Irritant.

Table 6-21 Accuracy of the BCOP Test Method in Distinguishing Substances Not Labeled as Irritants from All Other Irritant Classes, as Defined by the FHSA-20% Classification System,[1] with Discordant Chemical and Physical Classes Excluded

BCOP	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
Overall	194	83	161/194	95	139/147	47	22/47	53	25/47	5	8/147
Without Alcohols	177	81	144/177	94	125/133	43	19/44	57	25/44	6	8/133
Without Ketones	184	82	151/184	94	131/139	44	20/45	56	25/45	6	8/139
Without Solids	157	84	132/157	98	114/116	44	18/41	56	23/41	2	2/116
Without Alcohols and Ketones	168	80	135/168	94	118/126	40	17/42	60	25/42	6	8/126
Without Alcohols, Ketones, and Solids	132	81	107/132	98	94/96	36	13/36	64	23/36	2	2/96
Without Hydrocarbons	184	83	153/184	94	133/141	47	20/43	53	23/43	6	8/141

Abbreviations: BCOP = bovine corneal opacity and permeability; FHSA = Federal Hazardous Substances Act; N = number of substances included in this analysis/the total number of substances in the study; No. = data used to calculate the percentage.

[1] FHSA-20% classification system (2005): Not Labeled vs. Irritant.

Table 6-22 Accuracy of the BCOP Test Method in Distinguishing Substances Not Labeled as Irritants from All Other Irritant Classes, as Defined by the FHSA-67% Classification System,[1] with Discordant Chemical and Physical Classes Excluded

BCOP	N	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.
Overall	179	83	148/179	95	126/132	47	22/47	53	25/47	5	6/132
Without Alcohols	162	81	131/162	95	112/118	43	19/44	57	25/44	5	6/118
Without Ketones	170	82	139/170	95	119/125	44	20/45	56	25/45	5	6/125
Without Solids	144	83	120/144	99	102/103	44	18/41	56	23/41	1	1/103
Without Alcohols and Ketones	154	80	123/154	95	106/112	40	17/42	60	25/42	5	6/112
Without Alcohols, Ketones, and Solids	120	80	96/120	99	83/84	36	13/36	64	23/36	1	1/84
Without Hydrocarbons	170	83	141/170	95	121/127	47	20/43	53	23/43	5	6/127

Abbreviations: BCOP = bovine corneal opacity and permeability; FHSA = Federal Hazardous Substances Act; N = number of substances included in this analysis/the total number of substances in the study; No. = data used to calculate the percentage.

[1] FHSA-67% classification system (2005): Not Labeled vs. Irritant.

7.0 Bovine Corneal Opacity and Permeability Test Method Reliability

Assessment of test method reliability (intralaboratory repeatability and intra- and interlaboratory reproducibility) is essential to any evaluation of the performance of an alternative test method (ICCVAM 2003). Quantitative and qualitative evaluations of BCOP test method reliability have been conducted previously (ICCVAM 2006a).

However, additional qualitative analyses of test method reproducibility evaluated the extent of agreement of BCOP hazard classifications among the laboratories. Given that the performance of the BCOP test method was similar for the EPA and FHSA classification systems, additional reliability analyses were not conducted for the FHSA classification system.

7.1 Interlaboratory Reproducibility of Hazard Classification Category Using the GHS Classification System

Reliability analyses for the BCOP test method were evaluated for the following three studies: Balls et al. (1995), Gautheron et al. (1994), and Southee (1998).

Balls et al. (1995): Of 14 substances classified by the GHS as Not Labeled, 29% (4/14) were correctly identified, while two of four GHS Category 2B substances (50%) were correctly identified, 29% (4/14) substances classified as GHS Category 2A were correctly identified, and 77% (17/22) GHS Category 1 substances were correctly identified.

The five participating laboratories were in 100% agreement on the ocular irritancy classification when distinguishing Not Labeled substances from all other classes of 92% (55/60) substances (**Table 7-1**).

All five participating laboratories agreed on the classification of 88% (15/17) substances that were correctly identified as GHS Category 1, 0% (0/4) substances correctly classified as GHS Category 2A, 50% (1/2) substances correctly classified as GHS Category 2B, and 50% (2/4) substances correctly classified as GHS Not Classified (**Table 7-2**).

The extent of agreement between testing laboratories was greatest for substances identified from *in vivo* rabbit eye data as corrosives or severe irritants when compared to any other combination of *in vivo* and *in vitro* results. Eighty-eight percent (15/17) of the accurately identified severe substances were shown to have 100% classification agreement among testing laboratories (**Table 7-2**).

There was 100% agreement on the 10 false positive substances among the five laboratories.

Gautheron et al. (1994): Of 34 substances classified by the GHS as Not Classified, 38% (13/34) were correctly identified, while 0% (0/2) GHS Category 2B substances were correctly identified, 33% (1/3) substances classified as GHS Category 2A was correctly identified, and 75% (6/8) GHS Category 1 substances were correctly identified.

The 11–12 participating laboratories were in 100% agreement on the ocular irritancy classification when distinguishing substances not labeled as irritants from all other classes of 65% (34/52) substances (**Table 7-1**).

All 11–12 participating laboratories agreed on the classification of 67% (4/6) substances that were correctly identified as GHS Category 1, 0% (0/1) substances correctly classified as GHS Category 2A, and 0% (0/13) substance correctly classified as GHS Not Classified (**Table 7-2**).

The extent of agreement between testing laboratories was greatest for substances identified from *in vivo* rabbit eye data as corrosives or severe irritants when compared to any other combination of *in vivo* and *in vitro* results: 67% (4/6) of the accurately identified severe substances were shown to have 100% classification agreement among testing laboratories) (**Table 7-2**).

Of the 21 false positive substances, 90% (19/21) were shown to have 100% agreement among the 11-12 laboratories.

Southee (1998): Of 3 substances classified by the GHS as Not Classified, 67% (2/3) were correctly identified, while 50% (1/2) GHS Category 2B substances was correctly identified, 67% (2/3) of substances classified as GHS Category 2A were correctly identified, and 57% (4/7) of GHS Category 1 substances were correctly identified.

The three participating laboratories were in 100% agreement on the ocular irritancy classification when distinguishing substances not labeled as irritants from all other classes of 88% (14/16) substances (**Table 7-1**).

All three participating laboratories agreed on the classification of 100% (4/4) substances that were correctly identified as GHS Category 1, 50% (1/2) substances correctly classified as GHS Category 2A, 100% (1/1) substance correctly classified as GHS Category 2B, and 100% (2/2) substances correctly classified as GHS Not Classified (**Table 7-2**).

Regarding the 1 false positive substance, there was 100% agreement among the three laboratories.

Table 7-1 Reliability of the BCOP Test Method in Predicting Not Labeled Ocular Substances or Corrosives/Severe/Moderate/Mild Irritants, as Defined by the GHS Classification System,[1] by Study

Data Source	Classification (*In Vivo/ In Vitro*)	No. of Testing Labs	N	Substances with 100% Agreement Among Labs[2]	Substances with 91%-92% Agreement Among Labs	Substances with 82%-83% Agreement Among Labs	Substances with 80% Agreement Among Labs	Substances with 73%-75% Agreement Among Labs	Substances with 64%-67% Agreement Among Labs	Substances with 58%-60% Agreement Among Labs	Substances with ≤55% Agreement Among Labs
Balls et al. (1995)	+/+	5	40	38 (95%)	-	-	1 (3%)	-	-	-	1 (3%)
	+/-	5	0	-	-	-	-	-	-	-	-
	-/+	5	10	10 (100%)	-	-	-	-	-	-	-
	-/-	5	4	2 (50%)	-	-	1 (20%)	-	-	1 (20%)	-
	?/-	5	2	1 (50%)	-	-	-	-	-	-	1 (50%)
	?/+	5	4	4 (100%)	-	-	-	-	-	-	-
	Total		60	55 (92%)	-	-	2 (3%)	-	-	1 (2%)	2 (3%)
Gautheron et al. (1994)	+/+	11 12	13	11 (84%)	1 (8%)	-	-	1 (8%)	-	-	-
	+/-	11 12	0	-	-	-	-	-	-	-	-
	-/+	11 12	21	19 (90%)	-	-	-	2 (10%)	-	-	-
	-/-	11 12	13	-	-	1 (8%)	-	1 (8%)	2 (15%)	2 (15%)	7 (54%)
	?/-	11 12	1	-	-	-	-	-	1 (100%)	-	-
	?/+	11	4	4 (100%)	-	-	-	-	-	-	-
	Total		52	34 (65%)	1 (2%)	1 (2%)	-	4 (8%)	3 (6%)	2 (4%)	7 (13%)

Data Source	Classification (*In Vivo/ In Vitro*)	No. of Testing Labs	N	Substances with 100% Agreement Among Labs[2]	Substances with 91%-92% Agreement Among Labs	Substances with 82%-83% Agreement Among Labs	Substances with 80% Agreement Among Labs	Substances with 73%-75% Agreement Among Labs	Substances with 64%-67% Agreement Among Labs	Substances with 58%-60% Agreement Among Labs	Substances with ≤55% Agreement Among Labs
Southee (1998)	+/+	3	11	10 (91%)	-	-	-	-	-	-	1 (9%)
	+/-	3	1	-	-	-	-	-	-	-	1 (100%)
	-/+	3	1	1 (100%)	-	-	-	-	-	-	-
	-/-	3	2	2 (100%)	-	-	-	-	-	-	-
	?/-	3	0	-	-	-	-	-	-	-	-
	?/+	3	1	1 (100%)	-	-	-	-	-	-	-
	Total		16	14 (88%)	-	-	-	-	-	-	2 (12%)

Abbreviations: BCOP = bovine corneal opacity and permeability; GHS = Globally Harmonized System; N = number of substances.

A "+" indicates that the substance was assigned an overall classification of corrosive or a severe irritant (Category 1). A "-" indicates that the substance was assigned an overall classification of nonsevere irritant (Category 2A, 2B) or Not Labeled. A "?" indicates that, due to the lack of appropriate *in vivo* data (e.g., studies were terminated too early to assess reversibility of effects, insufficient dose volume), a GHS classification could not be made. See **Section 6.1** for a description of the rules followed to classify the ocular irritancy of test substances tested multiple times *in vitro*.

[1] GHS classification system (UN 2007).

[2] Number in parentheses indicates percentage of tested chemicals.

Table 7-2 Interlaboratory Variability of the BCOP Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the GHS Classification System,[1] by Study

Data Source	*In Vivo* Classification	Classification (*In Vitro*)	Number of Substances	Number of Testing Labs	Substances with 100% Agreement Among Laboratories (%)	Substances with 70%–95% Agreement Among Laboratories (%)	Substances with 60%–69% Agreement Among Laboratories (%)	Substances with <60% Agreement Among Laboratories (%)
Balls et al. (1995)	NL (14)	Actual	4	5	2 (50%)	1 (25%)	1 (25%)	-
		Over	10	5	10 (100%)	-	-	-
	2B (4)	Under	0	5	-	-	-	-
		Actual	2	5	1 (50%)	1 (50%)	-	-
		Over	2	5	1 (50%)	-	1 (50%)	-
	2A (14)	Under	2	5	2 (100%)	-	-	-
		Actual	4	5	-	1 (25%)	1 (25%)	2 (50%)
		Over	8	5	2 (25%)	3 (38%)	3 (38%)	-
	1 (22)	Under	5	5	3 (60%)	1 (20%)	1 (20%)	-
		Actual	17	5	15 (88%)	1 (6%)	1 (6%)	-
Gautheron et al. (1994)	NL (34)	Actual	13	11		3 (23%)	2 (15%)	8 (62%)
		Over	21	11	19 (90%)	1 (5%)	1 (5%)	-
	2B (2)	Under	0	11	-	-	-	-
		Actual	0	11	-	-	-	-
		Over	2	11	1 (50%)	1 (50%)	-	-
	2A (3)	Under	0	11	-	-	-	-
		Actual	1	11	-	1 (100%)	-	-
		Over	2	11	1 (50%)	1 (50%)	-	-
	1 (8)	Under	2	11	1 (50%)	1 (50%)	-	-
		Actual	6	11	4 (67%)	1 (17%)	-	1 (17%)

Data Source	*In Vivo* Classification	Classification (*In Vitro*)	Number of Substances	Number of Testing Labs	Substances with 100% Agreement Among Laboratories (%)	Substances with 70%–95% Agreement Among Laboratories (%)	Substances with 60%–69% Agreement Among Laboratories (%)	Substances with <60% Agreement Among Laboratories (%)
Southee (1998)	NL (3)	Actual	2	3	2 (100%)	-	-	-
		Over	1	3	1 (100%)	-	-	-
	2B (2)	Under	0	3	-	-	-	-
		Actual	1	3	1 (100%)	-	-	-
		Over	1	3	1 (100%)	-	-	-
	2A (3)	Under	0	3	-	-	-	-
		Actual	2	3	1 (50%)	1 (50%)	-	-
		Over	1	3	-	-	-	1 (100%)
	1 (7)	Under	3	3	3 (100%)	-	-	-
		Actual	4	3	4 (100%)	-	-	-

Abbreviations: BCOP = bovine corneal opacity and permeability; GHS = Globally Harmonized System; NL = Not Labeled as Irritant; 2B = mild irritant; 2A = moderate irritant; 1 = severe irritant.

[1] GHS classification system (UN 2007).

7.2 Interlaboratory Reproducibility of Hazard Classification Category Using the EPA Classification System

Balls et al. (1995): Both of the substances classified by the EPA as Category IV (100%) were correctly identified, while 29% (6/21) EPA Category III substances were correctly identified; 29% (4/14) EPA Category II substances were correctly identified, and 74% (14/19) EPA Category I substances were correctly identified.

The five participating laboratories were in 100% agreement on the ocular irritancy classification when assessing substances not labeled as irritants from all other classes of 93% (56/60) substances (**Table 7-3**).

All five participating laboratories agreed on the classification of 79% (11/14) substances that were correctly identified as EPA Category I, 0% (0/4) substances correctly classified as EPA Category II, 67% (4/6) substances correctly classified as EPA Category III, and 50% (1/2) substances correctly classified as EPA Category IV (**Table 7-4**).

When compared to any other combination of *in vivo* and *in vitro* results, the extent of agreement between testing laboratories was greatest for substances identified from *in vivo* rabbit eye data as corrosives or severe irritants. Of the accurately identified severe substances, 93% (13/14) were shown to have 80%–100% classification agreement among testing laboratories (**Table 7-4**).

Gautheron et al. (1994): Of 13 substances classified by the EPA as Category IV, 69% (9/13) were correctly identified, while 43% (9/21) EPA Category III substances were correctly identified, 25% (1/4 substances classified as EPA Category II was correctly identified, and 86% (6/7) EPA Category I substances were correctly identified.

The 11–12 participating laboratories were in 100% agreement on the ocular irritancy classification when assessing substances not labeled as irritants from all other classes of 65% (34/52) substances (**Table 7-3**).

All 11–12 participating laboratories agreed on the classification of 67% (4/6) substances that were correctly identified as EPA Category I, 0% (0/1) substances correctly classified as EPA Category II, 22% (2/9) substances correctly classified as EPA Category III, and 0% (0/9) substances correctly classified as EPA Category IV (**Table 7-4**).

All 4 false positive substances (100%) were shown to have 100% agreement among the 11–12 laboratories (**Table 7-4**).

Southee (1998): The one substance classified by the EPA as Category IV was correctly identified (100%), while 33% (2/6) EPA Category III substances were correctly identified, 50% (1/2) EPA Category II substances were correctly identified, and 50% (3/6) EPA Category I substances were correctly identified.

The three participating laboratories were in 100% agreement on the ocular irritancy classification when assessing substances not labeled as irritant from all other classes of 88% (14/16) substances (**Table 7-3**).

All three participating laboratories agreed on the classification of 100% (3/3) substances correctly identified as EPA Category I, 100% (1/1) substance correctly classified as EPA Category II, 100% (2/2) substances correctly classified as EPA Category III, and 100% (1/1) substance correctly classified as EPA Category IV (**Table 7-4**).

Table 7-3 Reliability of the BCOP Test Method in Predicting Not Labeled Ocular Substances or Corrosives/Severe/Moderate/Mild Irritants, as Defined by the EPA Classification System,[1] by Study

Data Source	Classification (*In Vivo/In Vitro*)	No. of Testing Labs	N	Substances with 100% Agreement Among Labs[2]	Substances with 91%–92% Agreement Among Labs	Substances with 82%–83% Agreement Among Labs	Substances with 80% Agreement Among Labs	Substances with 73% Agreement Among Labs	Substances with 64%–67% Agreement Among Labs	Substances with 58%–60% Agreement Among Labs	Substances with ≤55% Agreement Among Labs
Balls et al. (1995)	+/+	5	50	48 (96%)	-	-	1 (2%)	-	-	-	1 (2%)
	+/-	5	2	1 (50%)	-	-	-	-	-	1 (25%)	-
	-/+	5	0	-	-	-	-	-	-	-	-
	-/-	5	2	1 (50%)	-	-	1 (50%)	-	-	-	-
	?/-	5	1	1 (100%)	-	-	-	-	-	-	-
	?/+	5	5	5 (100%)	-	-	-	-	-	-	-
	Total		60	56 (93%)	-	-	2 (3%)	-	-	1 (2%)	1 (2%)
Gautheron et al. (1994)	+/+	11 12	31	27 (87%)	-	1 (3%)	-	3 (10%)	-	-	-
	+/-	11 12	4	-	-	1 (25%)	-	-	-	-	3 (75%)
	-/+	11 12	4	4 (100%)	-	-	-	-	-	-	-
	-/-	11 12	9	-	-	-	-	2 (22%)	2 (22%)	3 (34%)	2 (22%)
	?/-	11 12	1	-	-	-	-	-	1 (100%)	-	-
	?/+	11	3	3 (100%)	-	-	-	-	-	-	-
	Total		52	34 (65%)	-	2 (4%)	-	5 (9%)	3 (6%)	3 (6%)	5 (10%)

continued

Table 7-3 Reliability of the BCOP Test Method in Predicting Not Labeled Ocular Substances or Corrosives/Severe/Moderate/Mild Irritants, as Defined by the EPA Classification System,[1] by Study (continued)

Data Source	Classification (*In Vivo/In Vitro*)	No. of Testing Labs	N	Substances with 100% Agreement Among Labs[2]	Substances with 91%–92% Agreement Among Labs	Substances with 82%–83% Agreement Among Labs	Substances with 80% Agreement Among Labs	Substances with 73% Agreement Among Labs	Substances with 64%–67% Agreement Among Labs	Substances with 58%–60% Agreement Among Labs	Substances with ≤55% Agreement Among Labs
Southee (1998)	+/+	3	12	11 (92%)	-	-	-	-	-	-	1 (8%)
	+/-	3	2	1 (50%)	-	-	-	-	-	-	1 (50%)
	-/+	3	0	-	-	-	-	-	-	-	-
	-/-	3	1	1 (100%)	-	-	-	-	-	-	-
	?/-	3	0	-	-	-	-	-	-	-	-
	?/+	3	1	1 (100%)	-	-	-	-	-	-	-
	Total		16	14 (88%)	-	-	-	-	-	-	2 (12%)

Abbreviations: BCOP = bovine corneal opacity and permeability; EPA = U.S. Environmental Protection Agency; N = number of substances.

A "+" indicates that the substance was assigned an overall classification of corrosive or a severe irritant (Category I). A "-" indicates that the substance was assigned an overall classification of nonsevere irritant (Category II, III) or Not Labeled (category IV). A "?" indicates that, due to the lack of appropriate *in vivo* data (e.g., studies were terminated too early to assess reversibility of effects; insufficient dose volume), an EPA classification could not be made. See **Section 6.1** for a description of the rules followed to classify the ocular irritancy of test substances tested multiple times *in vitro*.

[1] EPA classification system (EPA 2003a).

[2] Number in parentheses indicates percentage of tested chemicals.

Table 7-4 Interlaboratory Variability of the BCOP Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the EPA Classification System,[1] by Study

Data Source	*In Vivo* Classification (No.)[2]	Classification (*In Vitro*)	Number of Substances	Number of Testing Laboratories	Substances with 100% Agreement Among Laboratories (%)	Substances with 80%–92% Agreement Among Laboratories (%)	Substances with 61%–79% Agreement Among Laboratories (%)	Substances with 50%–60% Agreement Among Laboratories (%)	Substances with <50% Agreement Among Laboratories (%)
Balls et al. (1995)	IV (2)	Actual	2	5	1 (50%)	1 (50%)	-	-	-
		Over	0	5	-	-	-	-	-
	III (21)	Under	2	5	1 (50%)	-	-	1 (50%)	-
		Actual	6	5	4 (67%)	1 (17%)	-	1 (17%)	-
		Over	13	5	7 (54%)	2 (15%)	-	4 (31%)	-
	II (14)	Under	2	5	2 (100%)	-	-	-	-
		Actual	4	5	-	1 (25%)	-	1 (25%)	2 (50%)
		Over	6	5	3 (50%)	1 (17%)	-	2 (33%)	-
	I (19)	Under	5	5	3 (60%)	1 (20%)	-	1 (20%)	-
		Actual	14	5	11 (79%)	2 (14%)	-	1 (7%)	-
Gautheron et al. (1994)	IV (13)	Actual	9	11/12	-	-	3 (33%)-	5 (56%)	1 (11%)
		Over	4	11/12	4 (100%)	-	-	-	-
	III (21)	Under	2	11/12	-	-	-	-	2 (100%)
		Actual	9	11/12	2 (22%)	4 (44%)	3 (33%)	-	-
		Over	10	11/12	8 (80%)	2 (20%)	-	-	-
	II (4)	Under	0	11/12	-	-	-	-	-
		Actual	1	11/12	-	1 (100%)	-	-	-
		Over	3	11/12	-	1 (33%)	2 (67%)	-	-
	I (7)	Under	2	11/12	1 (50%)	1 (50%)	-	-	-
		Actual	6	11/12	4 (67%)	1 (17%)	1 (17%)	-	-

continued

Table 7-4 Interlaboratory Variability of the BCOP Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the EPA Classification System,[1] by Study (continued)

Data Source	*In Vivo* Classification (No.)[2]	Classification (*In Vitro*)	Number of Substances	Number of Testing Laboratories	Substances with 100% Agreement Among Laboratories (%)	Substances with 80%–92% Agreement Among Laboratories (%)	Substances with 61%–79% Agreement Among Laboratories (%)	Substances with 50%–60% Agreement Among Laboratories (%)	Substances with <50% Agreement Among Laboratories (%)
Southee (1998)	IV (1)	Actual	1	3	1 (100%)	-	-	-	-
		Over	0	3	-	-	-	-	-
	III (6)	Under	1	3	1 (100%)	-	-	-	-
		Actual	2	3	2 (100%)	-	-	-	-
		Over	3	3	3 (100%)	-	-	-	-
	II (2)	Under	0	3	-	-	-	-	-
		Actual	1	5	1 (100%)	-	-	-	-
		Over	1	5	1 (100%)	-	-	-	-
	I (6)	Under	3	5	3 (100%)	-	-	-	-
		Actual	3	5	3 (100%)	-	-	-	-

Abbreviations: BCOP = bovine corneal opacity and permeability; EPA = U.S. Environmental Protection Agency; IV = Not Labeled as Irritant; III = mild irritant; II = moderate irritant; I = severe irritant.

[1] EPA classification system (EPA 2003a).

[2] Due to the lack of appropriate *in vivo* data (e.g., studies were terminated too early to assess reversibility of effects), a EPA classification could not be made for two substances. See **Section 6.1** for a description of the rules followed to classify the ocular irritancy of test substances tested multiple times *in vitro*.

7.3 Interlaboratory Reproducibility of Hazard Classification Category Using the EU Classification System

Balls et al. (1995): Of 16 substances classified by the EU as Not Labeled, 25% (4/16) were correctly identified, while 47% (7/15) EU R36 substances were correctly identified, and 74% (14/19) EU R41 substances were correctly identified.

The five participating laboratories were in 100% agreement on the ocular irritancy classification when assessing substances not labeled as irritant from all other classes of 93% (56/60) substances (**Table 7-5**).

All five participating laboratories agreed on the classification of 86% (12/14) substances that were correctly identified as EU R41, 29% (2/7) substances correctly classified as EU R36, and 50% (2/4) substances correctly classified as EU Not Labeled (**Table 7-6**).

When compared to any other combination of *in vivo* and *in vitro* results, the extent of agreement between testing laboratories was greatest for substances identified from *in vivo* rabbit eye data as corrosives or severe irritants. All (100%) of the accurately identified severe substances were shown to have 95%–100% classification agreement among testing laboratories (**Table 7-6**).

Of the 12 false positive substances, 100% (12/12) were shown to have 100% agreement among the 5 laboratories (**Table 7-6**).

Gautheron et al. (1994): Of 36 substances classified by the EU as Not Labeled, 36% (13/36) were correctly identified, while 50% (2/4) EU R36 substances were correctly identified, and 75% (6/8) EU R41 substances were correctly identified.

The 11–12 participating laboratories were in 100% agreement on the ocular irritancy classification when assessing non labeled substances from all other classes of 65% (34/52) substances (**Table 7-5**).

All 11–12 participating laboratories agreed on the classification of 67% (4/6) substances that were correctly identified as EU R41, 0% (0/2) substances correctly classified as EU R36, and 54% (7/13) substances correctly classified as EU Not Labeled (**Table 7-6**).

Of the 23 false positive substances, 91% (21/23) were shown to have 100% agreement among the 11-12 laboratories (**Table 7-6**).

Southee (1998): Of the 4 substances classified by the EU as Not Labeled, 50% (2/4) were correctly identified, while 50% (2/4) EU R36 substances were correctly identified, and 67% (4/6) EU R41 substances were correctly identified.

The three participating laboratories were in 100% agreement on the ocular irritancy classification when assessing substances not labeled as irritant from all other classes of 88% (14/16) substances (**Table 7-5**).

All three participating laboratories agreed on the classification of 100% (4/4) substances correctly identified as EU R41, 100% (3/3) substances correctly classified as EU R36, and 100% (2/2) substances correctly classified as EU Not Labeled (**Table 7-6**).

Of the 2 false positive substances, 50% (1/2) was shown to have 100% agreement among the three laboratories (**Table 7-6**).

Table 7-5 Reliability of the BCOP Test Method in Predicting Not Labeled Ocular Substances or Corrosives/Severe/Moderate Irritants, as Defined by the EU Classification System,[1] by Study

Data Source	Classification (*In Vivo/In Vitro*)	No. of Testing Labs	N	Substances with 100% Agreement Among Labs[2]	Substances with 91%–92% Agreement Among Labs	Substances with 82%–83% Agreement Among Labs	Substances with 80% Agreement Among Labs	Substances with 73% Agreement Among Labs	Substances with 64%–67% Agreement Among Labs	Substances with 58%–60% Agreement Among Labs	Substances with ≤55% Agreement Among Labs
Balls et al. (1995)	+/+	5	34	32 (94%)	-	-	1 (8%)	-	-	-	1 (8%)
	+/-	5	0	-	-	-	-	-	-	-	-
	-/+	5	12	12 (100%)	-	-	-	-	-	-	-
	-/-	5	4	2 (50%)	-	-	1 (25%)	-	-	1 (25%)	-
	?/-	5	1	1 (100%)	-	-	-	-	-	-	-
	?/+	5	9	9 (100%)	-	-	-	-	-	-	-
	Total		60	56 (93%)	-	-	2 (3%)	-	-	1 (2%)	1 (2%)
Gautheron et al. (1994)	+/+	11 12	12	10 (83%)	1 (8%)	-	-	1 (8%)	-	-	-
	+/-	11 12	0	-	-	-	-	-	-	-	-
	-/+	11 12	23	21 (91%)	-	-	-	2 (9%)	-	-	-
	-/-	11 12	13	-	-	1 (17%)	-	2 (16%)	2 (16%)	3 (23%)	5 (38%)
	?/-	11 12	1	-	-	-	-	-	1 (100%)	-	-
	?/+	11	3	3 (100%)	-	-	-	-	-	-	-
	Total		52	34 (65%)	1 (2%)	1 (2%)	-	5 (10%)	3 (6%)	3 (6%)	5 (10%)

continued

Table 7-5 Reliability of the BCOP Test Method in Predicting Not Labeled Ocular Substances or Corrosives/Severe/Moderate Irritants, as Defined by the EU Classification System,[1] by Study (continued)

Data Source	Classification (*In Vivo/In Vitro*)	No. of Testing Labs	N	Substances with 100% Agreement Among Labs[2]	Substances with 91%–92% Agreement Among Labs	Substances with 82%–83% Agreement Among Labs	Substances with 80% Agreement Among Labs	Substances with 73% Agreement Among Labs	Substances with 64%–67% Agreement Among Labs	Substances with 58%–60% Agreement Among Labs	Substances with ≤55% Agreement Among Labs
Southee (1998)	+/+	3	10	9 (90%)	-	-	-	-	-	-	1 (10%)
	+/-	3	1	-	-	-	-	-	-	-	1 (100%)
	-/+	3	2	2 (100%)	-	-	-	-	-	-	-
	-/-	3	2	2 (100%)	-	-	-	-	-	-	-
	?/-	3	0	-	-	-	-	-	-	-	-
	?/+	-	1	1 (100%)	-	-	-	-	-	-	-
	Total		16	14 (88%)	-	-	-	-	-	-	2 (12%)

Abbreviations: BCOP = bovine corneal opacity and permeability; EU = European Union; N = number of substances.

A "+" indicates that the substance was assigned an overall classification of corrosive or a severe irritant (Category R41). A "-" indicates that the substance was assigned an overall classification of nonsevere irritant (Category R36) or Not Labeled. A "?" indicates that, due to the lack of appropriate *in vivo* data (e.g., studies were terminated too early to assess reversibility of effects; insufficient dose volume), an EU classification could not be made. See Section 6.1 for a description of the rules followed to classify the ocular irritancy of test substances tested multiple times *in vitro*.

[1] EU classification system (EU 2001).

[2] Number in parentheses indicates percentage of tested chemicals.

Table 7-6 Interlaboratory Variability of the BCOP Test Method in Predicting Ocular Irritant Classes Compared to the *In Vivo* Rabbit Eye Test Method, as Defined by the EU Classification System,[1] by Study

Data Source	*In Vivo* Classification (No.)	Classification (*In Vitro*)	Number of Substances	Number of Testing Laboratories	Substances with 100% Agreement Among Laboratories (%)	Substances with 76%-95% Agreement Among Laboratories (%)	Substances with 50%-75% Agreement Among Laboratories (%)
Balls et al. (1995)	NL (16)	Actual	4	5	2 (50%)	1 (25%)	1 (25%)
		Over	12	5	12 (100%)	-	-
	R36 (15)	Under	0	5	-	-	-
		Actual	7	5	2 (29%)	2 (29%)	3 (42%)
		Over	8	5	3 (38%)	2 (24%)	3 (38%)
	R41 (19)	Under	5	5	3 (60%)	1 (20%)	1 (20%)
		Actual	14	5	12 (86%)	2 (14%)	-
Gautheron et al. (1994)	NL (36)	Actual	13	11/12	7 (54%)	2 (15%)	4 (31%)
		Over	23	11/12	21 (91%)	-	2 (9%)
	R36 (4)	Under	0	11/12	-	-	-
		Actual	2	11/12	-	1 (50%)	1 (50%)
		Over	2	11/12	1 (50%)	1 (50%)	-
	R41 (8)	Under	2	11/12	1 (50%)	1 (50%)	-
		Actual	6	11/12	4 (67%)	1 (17%)	1 (17%)
Southee (1998)	NL (4)	Actual	2	3	2 (100%)	-	-
		Over	2	3	1 (50%)	1 (50%)	-
	R36 (5)	Under	1	3	-	-	1 (100%)
		Actual	3	3	3 (100%)	-	-
		Over	1	3	1 (100%)	-	-
	R41 (6)	Under	2	3	2 (100%)	-	-
		Actual	4	3	4 (100%)	-	-

Abbreviations: BCOP = bovine corneal opacity and permeability; EU = European Union; NL = Not Labeled as Irritant; R36 = moderate/mild irritant; R41 = severe irritant.
[1] EU classification system (EU 2001).

8.0 Bovine Corneal Opacity and Permeability Test Method Data Quality

8.1 Adherence to National and International GLP Guidelines

The original evaluation of BCOP test method data quality is detailed in the previous BCOP BRD (ICCVAM 2006a). As indicated in Section 8.0 of the AMCP BRD (2008) submission, it could not be determined whether all of the *in vitro* data contained in the AMCP BRD were generated under full GLP compliance. Where possible, that information is contained in the spreadsheets that form the database from which the AMCP BRD was generated. All of the new *in vitro* data that were generated during the course of constructing the current ICCVAM 2010 BRD were conducted with full GLP compliance.

9.0 Reports in the Peer-Reviewed Literature

NICEATM located among the peer-reviewed literature a total of four BCOP studies published since the previous evaluation of the BCOP method for identification of ocular corrosives and severe irritants (ICCVAM 2006a) that contained BCOP data (Cater and Harbell 2006, 2008; Debbasch et al. 2005; Van Goethem et al. 2006). The four publications contained BCOP test method analyses; however, none of these publications included raw data and therefore were not added to the database.

In Debbasch et al. (2005), 12 makeup removers were tested both in the BCOP and in a clinical in-use test under ophthalmological control after their application to the external eyelid. The undiluted test product (750 μL) was pipetted onto the corneas and exposure conducted for 4 hours. Corneal opacity was determined using an adapted spectrophotometer and barrier disruption by fluorescein update using OD_{490} mm. *In vitro* scores were classified according to Gautheron et al. (1994) and Harbell and Curren (1998). However, no *in vivo* rabbit eye data were reported, and these data have not been obtained. For this reason, Debbasch et al. (2005) was not included in the BCOP performance analyses detailed in this BRD.

In Cater and Harbell (2006), surfactant-based "rinse-off" personal care formulations were tested in the BCOP test method using slight modifications of the BCOP protocol reported by Sina et al. (1995). Corneas were exposed to the test substances (750 μL) for 10, 30, or 60 minutes either undiluted or diluted in deionized water. Corneas were evaluated for opacity, fluorescein uptake, and histological alterations. No *in vivo* rabbit reference data were reported, and thus this study was not included in the BCOP performance analyses detailed in this BRD.

Van Goethem et al. (2006) tested 20 substances in the BCOP test method (7 compounds classified as GHS Not Classified and 13 GHS Category 1). These results were published in Vanparys et al. (1993) and Gautheron et al. (1994), which were included in the previous BCOP BRD (ICCVAM 2006a).

In Cater and Harbell (2008), the BCOP test method was used on four commercial and one unregistered body wash developed for children or as mild bath products. The purpose was to determine if the BCOP test method could be used as a prediction model for relative ranking of human eye responses under conditions of a standard human eye sting test to surfactant-based formulations. Test articles were prepared as 25% solutions in deionized water; 750 μL was applied to the corneas for a 30 minute exposure. Following exposure, opacity and fluorescein uptake were determined *in vitro*, but no *in vivo* rabbit eye data were reported.

10.0 Animal Welfare Considerations (Reduction, Refinement, and Replacement)

10.1 How the BCOP Test Method Will Reduce, Refine, or Replace Animal Use

ICCVAM promotes the scientific validation and regulatory acceptance of new methods that reduce, refine, or replace animal use where scientifically feasible. Refinement, reduction, and replacement are known as the "three Rs" of animal protection. These principles of humane treatment of laboratory animals are described as:

Reducing animal use through improved science and experimental design

Refining experimental procedures such that animal suffering is minimized

Replacing animal models with nonanimal procedures (e.g., *in vitro* technologies) where possible (Russell and Burch 1992)

The BCOP test method refines animal use. Because these animals are being humanely killed for nonlaboratory purposes, the testing procedure inflicts no additional pain or distress on animals. Substances that are identified as corrosive or severe irritants *in vitro* are excluded from *in vivo* testing. Furthermore, the ability to identify mild and moderate ocular irritants would eliminate the need for *in vivo* testing, thus sparing rabbits from the pain associated with these types of substances.

The BCOP test method can also reduce animal use because the test method utilizes animal species routinely raised as a food source in large numbers and thereby replaces laboratory animals. Additionally, with the ability to identify ocular corrosives and severe ocular irritants as well as mild and moderate ocular irritants from the *in vitro* method, the animals that would have been used in the *in vivo* rabbit eye test would be spared.

10.2 Requirement for the Use of Animals

Although cattle are required as a source of corneas for this *in vitro* test method, only cattle humanely killed for food or other nonlaboratory purposes are used as eye donors (i.e., no live animals are used in this test method).

11.0 Practical Considerations

Practical considerations for the BCOP method are detailed in the previous BCOP BRD (ICCVAM 2006a).

12.0 References

AMCP Background Review Document. 2008. Submission to ICCVAM: An *in vitro* approach of EPA toxicity labeling of anti-microbial cleaning products and annexes A–G (http://iccvam.niehs.nih.gov/methods/ocutox/antimicro/BRD.pdf).

Bailey PT, Freeman JJ, Phillips RD, Merrill JC. 2004. Validation of the BCOP assay as a predictor of ocular irritation of various petrochemical products. Poster presentation at the Society of Toxicology 2004 meeting (Baltimore, MD).

Balls M, Botham PA, Bruner LH, Spielmann H. 1995. The EC/HO international validation study on alternatives to the Draize eye irritation test. Toxicol In Vitro 9:871–929.

Berdasco N, Gilbert K, Lacher J, Mattsson J. 1996. Low rate of severe injury from dermal and ocular irritation tests and the validity of using fewer animals. J Am Coll Toxicol 15:177–193.

Calabrese EJ. 1983. Dermatotoxicity: predictive models. In: Principles of Animal Extrapolation. New York:Wiley Interscience.

Carpenter CP, Smyth HF. 1946. Chemical burns of the rabbit cornea. Am J Opthalmol 29:60–73.

Cater KC and Harbell JW. 2006. Prediction of eye irritation potential of surfactant-based rinse-off personal care formulations by the bovine corneal opacity and permeability (BCOP) assay. Cutan Ocul Toxicol 25:217–233.

Cater KC and Harbell JW. 2008. Comparison of *in vitro* eye irritation potential by bovine corneal opacity and permeability (BCOP) assay to erythema scores in human eye sting test of surfactant-based formulations. Cutan Ocul Toxicol 27:77–85.

CPSC. 2003. Commercial practices. Hazardous Substances and Articles; Administration and Enforcement Regulations. 16 CFR 1500.42.

Debbasch C, Ebenhahn C, Dami N, Pericoi M, Van den Berghe C, Cottin M, et al. 2005. Eye irritation of low-irritant cosmetic formulations: correlation of *in vitro* results with clinical data and product comparison. Food Chem Toxicol 43:155–165.

Draize J, Woodard G, Calvery H. 1944. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharmacol Exp Ther 82:377–390.

ECETOC. 1998. Eye Irritation – Reference Chemicals Data Bank. Technical Report No. 48(2). Brussels:European Centre for Ecotoxicology and Toxicology of Chemicals.

EEC. 1984. Commission Directive 84/449/EEC of 25 April 1984 adapting to technical progress for the sixth time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances (Acute Toxicity–Eye Irritation. Annex V, Part B.5). Official Journal of the European Communities L251:109.

EEC. 1991. Commission Directive 91/325/EEC of 1 March 1991 adapting to technical progress for the twelfth time Council Directive 67/548/EEC on the approximation of the laws, Regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. Official Journal of the European Communities L180:52–56.

EPA. 1998. Health Effects Test Guideline, OPPTS 870.2400 Acute Eye Irritation. EPA 712- C-98-195. Washington, DC:U.S. Environmental Protection Agency.

EPA. 2003a. Label Review Manual: 3rd ed. EPA737-B-96-001. Washington, DC:U.S. Environmental Protection Agency.

EPA. 2003b. Good Laboratory Practice Standards. Toxic Substances Control Act. 40 CFR 792.

administrative provisions relating to the classification, packaging and labelling of dangerous substances. Official Journal of the European Communities L255:1–333.

EU. 2004. Manual of Decisions for Implementation of the Sixth and Seventh Amendments to Directive 67/548/EEC on Dangerous Substances (Directives 79/831/EEC and 92/32/EEC). European Chemicals Bureau, IHCP, European Commission DG-JRC, report n. EUR 20519 EN, updated version of July 2004. Available: http://ecb.jrc.it/new-chemicals [accessed 14 October 2004].

FDA. 2003. Good laboratory practice for nonclinical laboratory studies. 21 CFR 58.

Federal Hazardous Substances Act (FHSA). 2005. Public Law 86-613. [74 Stat. 372].

Gautheron P, Giroux J, Cottin M, Audegond L, Morilla A, Mayordomo-Blanco L, et al. 1994. Interlaboratory assessment of the bovine corneal opacity and permeability (BCOP) assay. Toxicol In Vitro 8:381–392.

Harbell JW, Curren RD. 1998. The bovine corneal opacity and permeability assay: observations on assay performance. In Vitro Mol Toxicol 11:337–341.

ICCVAM. 2003. ICCVAM Guidelines for the Nomination and Submission of New, Revised, and Alternative Test Methods. NIH Publication No. 03-4508. Research Triangle Park, NC:National Institute of Environmental Health Sciences.

ICCVAM. 2006a. Background Review Document: Current Status of *In Vitro* Test Methods for Identifying Ocular Corrosives and Severe Irritants: Bovine Corneal Opacity and Permeability Test Method. NIH Publication No. 06-4512. Research Triangle Park, NC: National Institute of Environmental Health Sciences.

ICCVAM. 2006b. ICCVAM Test Method Evaluation Report: *In Vitro* Ocular Toxicity Methods for Identifying Severe Irritants and Corrosives. NIH Publication No. 07-4517. Research Triangle Park, NC: National Institute of Environmental Health Sciences.

ICCVAM. 2009. Independent Scientific Peer Review Panel Report: Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches. Research Triangle Park, NC:National Institute of Environmental Health Sciences.

ICCVAM Authorization Act of 2000. 2000. Public Law 106-545 [114 Stat. 2721].

Kay JH, Calandra JC. 1962. Interpretation of eye irritation tests. J Soc Cosmet Chem 13:281–289.

OECD. 1998. OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring Number 1: OECD Principles of Good Laboratory Practice (as revised in 1997). ENV/MC/CHEM(98)17. Paris:Organisation for Economic Co-operation and Development.

OECD. 2002. Test Guideline 405. Acute eye irritation/corrosion, adopted April 24, 2002. In: OECD Guidelines for Testing of Chemicals. Paris:Organisation for Economic Co-operation and Development.

OECD. 2005. OECD Series on Testing and Assessment Number 34. Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment. Paris:Organisation for Economic Co-operation and Development. Available: http://appli1.oecd.org/olis/2005doc.nsf/linkto/env-jm-mono(2005)14.

Russell WMS, Burch RL. 1992. The principles of humane experimental technique. 14th ed. Herts, England:Universities Federation for Animal Welfare.

Sina JF, Galer DM, Sussman RG, Gautheron PD, Sargent EV, Leong B, et al. 1995. A collaborative evaluation of seven alternatives to the Draize eye irritation test using pharmaceutical intermediates. Fundam Appl Toxicol 26:20–31.

Southee JA. 1998. Evaluation of the Prevalidation Process. Part 2, final report. Volume 2. The Bovine Corneal Opacity and Permeability (BCOP) Assay. European Community Contract No. 11279-95-10F 1ED ISP GB.

Swanson JE, Harbell JW. 2000. Evaluating the eye irritancy potential of ethanolic test materials with the bovine corneal opacity and permeability assay. The Toxicologist 54:188–189.

Swanson JE, Lake LK, Donnelly TA, Harbell JW, Huggins J. 1995. Prediction of ocular irritancy of full-strength cleaners and strippers by tissue equivalent and bovine corneal assays. J Toxicol Cutaneous Ocul Toxicol 14:179–195.

United Nations (UN) (2007). Globally Harmonized System of Classification and Labelling of Chemicals (GHS), Second revised edition, UN New York and Geneva, 2007. Available at: http://www.unece.org/trans/danger/publi/ghs/ghs_rev02/02files_e.html.

Van Goethem F, Adriaens E, Alepee N, Straube F, De Wever B, Cappadoro M, et al. 2006. Prevalidation of a new *in vitro* reconstituted human cornea model to assess the eye irritating potential of chemicals. Toxicol in Vitro 20:1–17.

Vanparys P, Deknudt G, Sysmans M, Teuns G, Coussement W, Van Cauteren H. 1993. Evaluation of the bovine corneal opacity-permeability assay as an in vitro alternative to the Draize eye irritation test. Toxicol In Vitro 7:471–476.

13.0 Glossary[2]

Accuracy:[3] (a) The closeness of agreement between a test method result and an accepted reference value. (b) The proportion of correct outcomes of a test method. It is a measure of test method performance and one aspect of "relevance." The term is often used interchangeably with *concordance* (see also *two-by-two table*). Accuracy is highly dependent on the prevalence of positives in the population being examined.

Assay:[3] The experimental system used. Often used interchangeably with *test* and *test method*.

Benchmark control: A sample containing all components of a test system and treated with a known substance (i.e., the benchmark substance) to induce a known response. The sample is processed with test substance-treated and other control samples to compare the response produced by the test substance to the benchmark substance to allow for an assessment of the sensitivity of the test method to assess a specific chemical class or product class.

Benchmark substance: A substance used as a standard for comparison to a test substance. A benchmark substance should have the following properties:

- a consistent and reliable source(s)
- structural and functional similarity to the class of substances being tested
- known physical/chemical characteristics
- supporting data on known effects
- known potency in the range of the desired response

Blepharitis: Inflammation of the eyelids.

Bulbar conjunctiva: The portion of the conjunctiva that covers the outer surface of the eye.

Chemosis: A form of eye irritation in which the membranes that line the eyelids and surface of the eye (*conjunctiva*) become swollen.

Classification system: An arrangement of quantified results or data into groups or categories according to previously established criteria.

Coded substances: Substances labeled by code rather than name so that they can be tested and evaluated without knowledge of their identity or anticipation of test results. Coded substances are used to avoid intentional or unintentional bias when evaluating laboratory or test method performance.

Coefficient of variation: A statistical representation of the precision of a test. It is expressed as a percentage and is calculated as follows:

$$\left(\frac{standard\ deviation}{mean}\right) \times 100\%$$

Concordance:[3] The proportion of all substances tested that are correctly classified as positive or negative. It is a measure of test method performance and one aspect of *relevance*. The term is often used interchangeably with *accuracy* (see also *two-by-two table*). Concordance is highly dependent on the prevalence of positives in the population being examined.

---

[2] The definitions in this Glossary are restricted to their uses with respect to the Draize rabbit eye test method and the BCOP test method.

[3] Definition used by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM 2003).

Conjunctiva: The mucous membrane that lines the inner surfaces of the eyelids and folds back to cover the front surface of the eyeball, except for the central clear portion of the outer eye (the cornea). The conjunctiva is composed of three sections: palpebral conjunctiva, bulbar conjunctiva, and fornix.

Conjunctival sac: The space located between the eyelid and the conjunctiva-covered eyeball. Substances are instilled into the sac to conduct an *in vivo* eye test.

Cornea: The transparent part of the coat of the eyeball that covers the iris and pupil and admits light to the interior.

Corneal opacity: Measurement of the extent of opaqueness of the cornea following exposure to a test substance. Increased corneal opacity is indicative of damage to the cornea. Opacity can be evaluated subjectively as done in the Draize rabbit eye test, or objectively with an instrument such as an opacitometer.

Corneal permeability: Quantitative measurement of damage to the corneal epithelium by a determination of the amount of sodium fluorescein dye that passes through all corneal cell layers.

Corrosion: Destruction of tissue at the site of contact with a substance.

Corrosive: A substance that causes irreversible tissue damage at the site of contact.

Endpoint:[3] The biological process, response, or effect assessed by a test method.

False negative:[3] A substance incorrectly identified as negative by a test method.

False negative rate:[3] The proportion of all positive substances falsely identified by a test method as negative (see *two-by-two table*). It is one indicator of test method accuracy.

False positive:[3] A substance incorrectly identified as positive by a test method.

False positive rate:[3] The proportion of all negative substances that are falsely identified by a test method as positive (see *two-by-two table*). It is one indicator of test method accuracy.

Fibrous tunic: The outer of the three membranes of the eye, comprising the cornea and the sclera; also called *tunica fibrosa oculi*.

Globally Harmonized System (GHS): A classification system presented by the United Nations that provides (a) a harmonized criteria for classifying substances and mixtures according to their health, environmental and physical hazards, and (b) harmonized hazard communication elements, including requirements for labeling and safety data sheets.

Good Laboratory Practices (GLP):[3] Regulations promulgated by the U.S. Food and Drug Administration and the U.S. Environmental Protection Agency, and principles and procedures adopted by the Organization for Economic Cooperation and Development and Japanese authorities that describe record keeping and quality assurance procedures for laboratory records that will be the basis for data submissions to national regulatory agencies.

Hazard:[3] The potential for an adverse health or ecological effect. A hazard potential results only if an exposure occurs that leads to the possibility of an adverse effect being manifested.

Interlaboratory reproducibility:[3] A measure of whether different qualified laboratories using the same protocol and test substances can produce qualitatively and quantitatively similar results. Interlaboratory reproducibility is determined during the prevalidation and validation processes and indicates the extent to which a test method can be transferred successfully among laboratories.

Intralaboratory repeatability:[3] The closeness of agreement between test results obtained within a single laboratory when the procedure is performed on the same substance under identical conditions within a given time period.

Intralaboratory reproducibility:[3] The first stage of validation; a determination of whether qualified people within the same laboratory can successfully replicate results using a specific test protocol at different times.

In vitro: In glass. Refers to assays that are carried out in an artificial system (e.g., in a test tube or petri dish) and typically use single-cell organisms, cultured cells, cell-free extracts, or purified cellular components.

***In vitro* irritancy score:** An empirically derived formula used in the BCOP assay whereby the mean opacity and mean permeability values for each treatment group are combined into a single *in vitro* score for each treatment group. The *in vitro* irritancy score = mean opacity value + (15 x mean permeability value).

In vivo : In the living organism. Refers to assays performed in multicellular organisms.

Iris: The contractile diaphragm perforated by the pupil and forming the colored portion of the eye.

Negative control: An untreated sample containing all components of a test system, except the test substance solvent, which is replaced with a known nonreactive material, such as water. This sample is processed with test substance-treated samples and other control samples to determine whether the solvent interacts with the test system.

Negative predictivity:[3] The proportion of correct negative responses among substances testing negative by a test method (see *two-by-two table*). It is one indicator of test method accuracy. Negative predictivity is a function of the sensitivity of the test method and the prevalence of negatives among the substances tested.

Neuroectodermal tunic: The innermost of three membranes of the eye, comprising the retina.

Nictating (nictitating) membrane: The membrane that moves horizontally across the eye in some animal species (e.g., rabbit, cat) to provide additional protection in particular circumstances. It may be referred to as the *third eyelid*.

Nonsevere irritant: (a) A substance that causes tissue damage in the eye following application to the anterior surface of the eye; the tissue damage is reversible within 21 days of application and the observed adverse effects in the eye are less severe than observed for a severe irritant. (b) Substances that are classified as GHS Category 2A or 2B; EPA Category II, III, or IV; or EU R36 ocular irritants.

Not Labeled: (a) A substance the produces no changes in the eye following application to the anterior surface of the eye. (b) Substances that are not classified as GHS Category 1, 2A, or 2B; or EU R41 or R36 ocular irritants.

Ocular: Of or relating to the eye.

Ocular corrosive: A substance that causes irreversible tissue damage in the eye following application to the anterior surface of the eye.

Ocular irritant: A substance that produces a reversible change in the eye following application to the anterior surface of the eye.

Opacitometer: An instrument used to measure *corneal opacity* by quantitatively evaluating light transmission through the cornea. The instrument has two compartments, each with its own light source and photocell. One compartment is used for the treated cornea, while the other is used to calibrate and zero the instrument. The difference between photocell signals in the two compartments is measured electronically as a change in voltage and is displayed digitally, generating numerical opacity values with arbitrary units.

Palpebral conjunctiva: The part of the conjunctiva that covers the inner surface of the eyelids.

Pannus: A specific type of corneal inflammation that begins within the conjunctiva and with time spreads to the cornea. Also referred to as *chronic superficial keratitis*.

Performance:[3] The accuracy and reliability characteristics of a test method (see *accuracy*, *reliability*).

pH: A measure of the acidity or alkalinity of a solution. pH 7.0 is neutral; higher pHs are alkaline, lower pHs are acidic.

Positive control: A sample containing all components of a test system and treated with a substance known to induce a positive response, which is processed with the test substance-treated and other control samples to demonstrate the sensitivity of each experiment and to allow for an assessment of variability in the conduct of the assay over time.

Positive predictivity:[3] The proportion of correct positive responses among substances testing positive by a test method (see *two-by-two table*). It is one indicator of test method accuracy. Positive predictivity is a function of the sensitivity of the test method and the prevalence of positives among the substances tested.

Prevalence:[3] The proportion of positives in the population of substances tested (see *two-by-two table*).

Protocol:[3] The precise, step-by-step description of a test method, including a listing of all necessary reagents, criteria, and procedures for evaluation of the test data.

Quality assurance:[3] A management process by which adherence to laboratory testing standards, requirements, and record keeping procedures is assessed independently by individuals other than those performing the testing.

Reduction alternative:[3] A new or modified test method that reduces the number of animals required.

Reference test method:[3] The accepted *in vivo* test method used for regulatory purposes to evaluate the potential of a test substance to be hazardous to the species of interest.

Refinement alternative:[3] A new or modified test method that refines procedures to lessen or eliminate pain or distress in animals, or enhances animal well-being.

Relevance:[3] The extent to which a test method correctly predicts or measures the biological effect of interest in humans or another species of interest. Relevance incorporates consideration of the *accuracy* or *concordance* of a test method.

Reliability:[3] A measure of the degree to which a test method can be performed reproducibly within and among laboratories over time. It is assessed by calculating intra- and interlaboratory reproducibility and intralaboratory repeatability.

Replacement alternative:[3] A new or modified test method that replaces animals with nonanimal systems or one animal species with a phylogenetically lower one (e.g., a mammal with an invertebrate).

Reproducibility:[3] The consistency of individual test results obtained in a single laboratory (intralaboratory reproducibility) or in different laboratories (interlaboratory reproducibility) using the same protocol and test substances (see intra- and inter-laboratory reproducibility).

Sclera: The tough, fibrous tissue that extends from the cornea to the optic nerve at the back of the eye.

Sensitivity:[3] The proportion of all positive substances that are classified correctly as positive in a test method. It is a measure of test method accuracy (see *two-by-two table*).

Secondary bacterial keratitis: Inflammation of the cornea that occurs secondary to another insult that compromised the integrity of the eye.

Severe irritant: (a) A substance that causes tissue damage in the eye following application to the anterior surface of the eye that is not reversible within 21 days of application or causes serious physical

decay of vision. (b) Substances that are classified as EPA Category I, GHS Category 1, or EU R41 ocular irritants.

Solvent control: An untreated sample containing all components of a test system, including the solvent that is processed with the test substance-treated and other control samples to establish the baseline response for the samples treated with the test substance dissolved in the same solvent. When tested with a concurrent negative control, this sample also demonstrates whether the solvent interacts with the test system.

Specificity:[3] The proportion of all negative substances that are classified correctly as negative in a test method. It is a measure of test method accuracy (see *two-by-two table*).

Test:[2] The experimental system used; used interchangeably with *test method* and *assay*.

Test method:[3] A process or procedure used to obtain information on the characteristics of a substance or agent. Toxicological test methods generate information regarding the ability of a substance or agent to produce a specified biological effect under specified conditions. Used interchangeably with *test* and *assay*. See also *validated test method* and *reference test*.

Test method component: Structural, functional, and procedural elements of a test method that are used to develop the test method protocol. These components include unique characteristics of the test method, critical procedural details, and quality control measures.

Tiered testing: A testing strategy where all existing information on a test substance is reviewed, in a specified order, prior to *in vivo* testing. If the irritancy potential of a test substance can be assigned, based on the existing information, no additional testing is required. If the irritancy potential of a test substance cannot be assigned, based on the existing information, a step-wise animal testing procedure is performed until an unequivocal classification can be made.

Toxic keratoconjunctivitis: Inflammation of the cornea and conjunctiva due to contact with an exogenous agent. Used interchangeably with *contact keratoconjunctivitis*, *irritative keratoconjunctivitis* and *chemical keratoconjunctivitis*.

Transferability:[3] The ability of a test method or procedure to be accurately and reliably performed in different, competent laboratories.

Two-by-two table:[3] The two-by-two table can be used for calculating accuracy (concordance) ([a+d]/[a+b+c+d]), negative predictivity (d/[c+d]), positive predictivity (a/[a+b]), prevalence ([a+c]/[a+b+c+d]), sensitivity (a/[a+c]), specificity (d/[b+d]), false positive rate (b/[b+d]), and false negative rate (c/[a+c]).

		New Test Outcome		
		Positive	Negative	Total
Reference Test Outcome	Positive	a	c	a + c
	Negative	b	d	b + d
	Total	a + b	c + d	a + b + c + d

Uvea tract: The middle of three membranes of the eye, comprising the iris, ciliary body, and choroid. Also referred to as the *vascular tunic*.

Validated test method:[3] An accepted test method for which validation studies have been completed to determine the relevance and reliability of this method for a specific proposed use.

Validation:[3] The process by which the reliability and relevance of a procedure are established for a specific purpose.

Vascular tunic: The middle of three membranes of the eye, comprising the iris, ciliary body, and choroid. Also referred to as the *uvea*.

Weight of evidence (process): The strengths and weaknesses of a collection of information are used as the basis for a conclusion that may not be evident from the individual data.

This page intentionally left blank

Annex I

Characterization of Substances Tested in the BCOP Test Method

Annex I-1
Chemical and Product Classes of Substances Tested in the BCOP Assay.....C-109

Annex I-2
Components of Formulations Tested in Gettings et al. (1996).....C-123

Annex I-3
Components of Formulations Tested in Swanson et al. (1995).....C-135

Annex I-4
Components of Formulations Tested in Swanson and Harbell (2000).....C-141

Annex I-5
Components of AMCP Formulations Tested C-145

This page intentionally left blank

Annex I-1

Chemical and Product Classes of Substances Tested in the BCOP Assay

This page intentionally left blank

Chemical and Product Classes of Substances Tested in the BCOP Assay

Substance	CASRN[1]	Chemical Class	Product Class
1-1 (#1)	-	Formulation	Insect repellent
1-2 (#2)	-	Formulation	Insect repellent
1-3 (#3)	-	Formulation	Insect repellent
2-4 (#4)	-	Formulation	Insect repellent
2-7 (#7)	-	Formulation	Insect repellent
2-8 (#8)	-	Formulation	Insect repellent
Acetone	67-64-1	Ketone	Solvent; Antiseptic; Chemical intermediate; Raw material
Alkyl phosphoric acid ester/amine salt	-	Organic salt, Ester, Amine	Petroleum product
All Purpose Cleaner (#5)	-	Formulation	Cleaner
All Purpose Cleaner (#7)	-	Formulation	Cleaner
Allyl alcohol	107-18-6	Alcohol	Pesticide
Aluminum hydroxide	21645-51-2	Alkali, Aluminum compound	Chemical intermediate, Dessicant
2-Aminophenol	95-55-6		Chemical intermediate
Ammonium nitrate	6484-52-2	Inorganic salt, Onium compound	Fertilizer; Chemical intermediate; Industrial explosive
Amway all fabric bleach	-	Formulation	Detergent
Amway automatic dishwashing compound for soft water	-	Formulation	Detergent
Amway automatic dishwashing compound, standard formula	-	Formulation	Detergent
Amway concrete floor cleaner	-	Formulation	Cleaner
Amway Dish Drops dishwashing liquid	-	Formulation	Detergent
Amway dry chlorine bleach	-	Formulation	Bleach

Chemical and Product Classes of Substances Tested in the BCOP Assay

Substance	CASRN[1]	Chemical Class	Product Class
Amway fabric softener	-	Formulation	Fabric softener
Amway Kool Wash delicate fabric detergent	-	Formulation	Detergent
Amway LOC all purpose cleaner	-	Formulation	Cleaner
Amway prewash liquid	-	Formulation	Detergent
Amway Pursue disinfectant cleaner	-	Formulation	Cleaner
Amway Redu dye stain remover	-	Formulation	Stain remover
Amway SA8 laundry liquid	-	Formulation	Detergent
Amway SA8 limited phos laundry powder	-	Formulation	Detergent
Anthracene	120-12-7	Polycyclic	Dye manufacturing agent
Anti-Dandruff Shampoo (HZY) 100%	-	Formulation	Surfactant-containing formulation
Aromatic hydrocarbon #1	-	Hydrocarbon (cyclic)	Solvent/industrial chemical; Petrochemical product
Aromatic hydrocarbon #2	-	Hydrocarbon (cyclic)	Solvent/industrial chemical; Petrochemical product
Aryl phosponates	-	Not classified	Lubricant additive; Petrochemical product
L-Aspartic acid	70-47-3	Amino acid	Organic intermediate; Fungicides; Germicides
Baby Shampoo No. 1 (HZP)	-	Formulation	Surfactant-containing formulation
Baby Shampoo No. 2 (HZF)	-	Formulation	Surfactant-containing formulation
Bathroom Cleaner (#6)	-	Formulation	Cleaner
Benchmark-Group 1 (#12)	-	Formulation	Insect repellent
Benchmark-Group 2 (#13)	-	Formulation	Insect repellent
Benzalkonium chloride (100%)	8001-54-5	Onium compound	Surfactant (cationic); Bactericide; Fungicide; Preservative

Chemical and Product Classes of Substances Tested in the BCOP Assay

Substance	CASRN[1]	Chemical Class	Product Class
Benzalkonium chloride (1 %)	8001-54-5	Inorganic salt, Onium compound	Fertilizer; Chemical intermediate; Industrial explosive
Benzalkonium chloride (10%)	8001-54-5	Onium compound	Surfactant (cationic); Bactericide; Fungicide; Preservative
Benzalkonium chloride (5%)	8001-54-5	Onium compound	Surfactant (cationic); Bactericide; Fungicide; Preservative
Benzethonium chloride	121-54-0	Amine, Onium compound	Bactericide
Benzoyl-L-tartaric acid	2743-38-6	Carboxylic acid, Ester	Optical resolution agent
Betaine monohydrate	590-47-6	Amino acid, Onium compound	Not classified
BRIJ-35	9002-92-0	Alcohol	Emulsifier
4-Bromophenetole	589-10-6	Ether	Not classified
Bubble Bath (HZK) 100%	-	Formulation	Surfactant-containing formulation
n-Butanol	71-36-3	Ketone	Solvent; Synthetic flavor; Drycleaning
2-Butoxyethanol	111-76-2	Alcohol	Solvent
Butyl acetate	123-86-4	Ester	Solvent; Synthetic flavor ingredient
Butyl cellosolve	111-76-2	Alcohol, Ester	Solvent
Butyrolactone	96-48-0	Lactone, Heterocycle	Synthetic intermediate; Solvent
gamma-Butyrolactone	96-48-0	Heterocyclic, Lactone	Synthetic intermediate; Solvent
Captan 90 concentrate	133-06-2	Imide, Organic sulfur compound	Pesticide
4-Carboxybenzaldehyde	619-66-9	Carboxylic acid, Aldehyde	Not classified
Carboxylic acid amides	-	Formulation	Lubricant additive; Petrochemical product
Cetylpyridinium bromide (0.1%)	140-72-7	Heterocyclic, Onium compound	Surfactant (cationic); Germicide; Laboratory reagent
Cetylpyridinium bromide (1%)	140-72-7	Surfactant, cationic	Germicide; Laboratory reagent

Chemical and Product Classes of Substances Tested in the BCOP Assay

Substance	CASRN[1]	Chemical Class	Product Class
Cetylpyridinium bromide (10%)	140-72-7	Heterocyclic, Onium compound	Surfactant (cationic); Germicide; Laboratory reagent
Cetylpyridinium bromide (6%)	140-72-7	Heterocyclic, Onium compound	Surfactant (cationic); Germicide; Laboratory reagent
Chlorhexidine	55-56-1	Amine/Amidine	Disinfectant; Mouthwash; Anti-infective agent
2-Chloro-2,4,4-trimethylpentane	-	Hydrocarbon (halogenated)	Solvent/industrial chemical; Petrochemical product
Clarified slurry oil	-	Hydrocarbon (cyclic)	Petrochemical product
Cleaner/Degreaser (#13)	-	Formulation	Cleaner
Cleansing Gel (HZQ) 100%	-	Formulation	Surfactant-containing formulation
Cutting fluid (conc.) #1	-	Formulation	Cutting fluid; Petrochemical product
Cutting fluid (conc.) #2	-	Formulation	Cutting fluid; Petrochemical product
Cyclohexanol	108-93-0	Alcohol	Solvent; Chemical intermediate
Cyclohexanone	108-94-1	Ketone, Hydrocarbon (cyclic)	Solvent, Chemical intermediate
Degreaser (#16)	-	Formulation	Degreaser
Deoxycholic acid, sodium salt	302-95-4	Alcohol, Carboxylic acid (salt)	Detergent/Surfactant, Chemical intermediate
Diacetone alcohol	123-42-2	Ketone, Alcohol	Solvent
Dibenzyl phosphate	1623-08-1	Ester, Organophosphorus compound	Not classified
2,6-Dichlorobenzoyl chloride	4659-45-4	Acyl halide	Anti-infective; Anti-fungal; Preservative
2,4-Dichloro-5-sulfamoylbenzoic acid	2736-23-4	Amide, Organic sulfur compound	Intermediate for pharmaceticals, pesticides, perfumes
2,4-Difluoronitrobenzene	446-35-5	Acyl halide	Anti-infective; Anti-fungal; Preservative
1,3-Diisopropylbenzene	99-62-7	Hydrocarbon (cyclic)	Not classified
Dimethylbiguanide	657-24-9	Amidine	Pharmaceutical

Chemical and Product Classes of Substances Tested in the BCOP Assay

Substance	CASRN[1]	Chemical Class	Product Class
2,2-Dimethylbutanoic acid	595-37-9	Carboxylic acid	Pharmaceutical metabolite
2,5-Dimethylhexanediol	110-03-2	Alcohol	Intermediate for pharmaceticals, pesticides, perfumes
Dimethyl sulfoxide	67-68-5	Organic sulfur compound	Solvent
Dodecane	112-40-3	Hydrocarbon (acyclic)	Not classified
EDTA, di-potassium salt	25102-12-9	Amine, Carboxylic acid (salt)	Chelator
Ethanol	64-17-5	Alcohol	Solvent; Beverages; Antifreeze agent
Ethanol (#14)	64-17-5	Alcohol	Solvent
2-Ethoxyethanol	110-80-5	Alcohol	Solvent
Ethyl acetate	141-78-6	Ester	Solvent; Synthetic flavoring
Ethyl acetoacetate	141-97-9	Carboxylic acid, Ketone	Chemical intermediate, Flavoring agent
2-Ethylhexanol	104-76-7	Alcohol	Intermediate for pharmaceticals, pesticides, perfumes
2-Ethyl-1-hexanol	104-76-7	Alcohol	Solvent; Plasticizer
Ethylhexyl acid phosphate ester	-	Ester, Carboxylic acid	Lubricant additive; Petrochemical product
5-Ethylidene-2-norbornene	16219-75-3	Not classified	Solvent/industrial chemical; Petrochemical product
Ethyl-2-methylacetoacetate	609-14-3	Ketone, Ester	Not classified
3-Ethyltoluene	620-14-4	Hydrocarbon (cyclic)	Not classified
Ethyl trimethyl acetate	3938-95-2	Ester	Solvent
Eye Make-Up Remover (HZH) 100%	-	Formulation	Surfactant-containing formulation
Facial Cleaning Foam (HZR) 25%	-	Formulation	Surfactant-containing formulation
Facial Cleanser (HZZ) 100%	-	Formulation	Surfactant-containing formulation

Chemical and Product Classes of Substances Tested in the BCOP Assay

Substance	CASRN[1]	Chemical Class	Product Class
Floor Cleaner (#10)	-	Formulation	Cleaner
Floor Cleaner (#2)	-	Formulation	Cleaner
Floor Stripper (#14)	-	Formulation	Floor stripper
Floor Stripper (#17)	-	Formulation	Floor stripper
Floor Stripper (#18)	-	Formulation	Floor stripper
Foam Bath (HZL) 100%	-	Formulation	Surfactant-containing formulation
Fomesafen	72128-02-0	Imide, Ether, Nitro compound	Pesticide
Furan	110-00-9	Heterocyclic	Chemical intermediate
Gel Cleanser (HZE) 100%	-	Formulation	Surfactant-containing formulation
General Cleaner (#11)	-	Formulation	Cleaner
General Cleaner (#12)	-	Formulation	Cleaner
Glass Cleaner (#19)	-	Formulation	Cleaner
Gluconolactone	90-80-2	Carboxylic acid, Lactone, Carbohydrate	Food additive
DL-Glutamic acid	19285-83-7	Amino acid	Not classified
Glycerol	56-81-5	Alcohol	Solvent; Plasticizer; Lubricant; Emollient; Drug vehicle
3-Glycidoxypropyltrimethoxysilane	2530-83-8	Organosilicon compound	Adhesive
Hand Soap (HZU) 25%	-	Formulation	Surfactant-containing formulation
Heavy Duty Cleaner (#15)	-	Formulation	Cleaner
Heavy Duty Cleaner/Degreaser (#9)	-	Formulation	Cleaner
Hexadecyltrimethylammonium bromide	57-09-0	Organic salt, Onium compound	Agricultural chemical; Germicide; Drug/Therapeutic agent

Chemical and Product Classes of Substances Tested in the BCOP Assay

Substance	CASRN[1]	Chemical Class	Product Class
1,5-Hexadiene	592-42-7	Hydrocarbon (acyclic)	Not classified
Hexane	110-54-3	Hydrocarbon (acyclic)	Solvent
n-Hexanol	111-27-3	Alcohol	Solvent; Chemical intermediate; Synthetic flavor ingredient
Imidazole	288-32-4	Heterocyclic	Anti-fungal; Enzyme inhibitor
Iminodibenzyl	494-19-9	Heterocyclic	Personal care product
Isobutanol	78-83-1	Alcohol	Solvent; Chemical intermediate; Flavor ingredient
Isopropanol	67-63-0	Alcohol	Solvent; Aerosol formulations (ingredient)
N-Lauroylsarcosine, sodium salt	7631-98-3	Amide, Amino acid (salt)	Surfactant (anionic)
Laurylsulfobetaine	14933-08-5	Amine, Onium compound	Detergent, Surfactant (zwitterionic)
Liquid Soap No. 2 (HZW) 25%	-	Formulation	Surfactant-containing formulation
Liquid Soap No. 1 (HZB) 25%	-	Formulation	Surfactant-containing formulation
Magnesium carbonate	56378-72-4	Inorganic salt	Chemical intermediate
Maneb	12427-38-2	Amine/Amidine, Organic salt, Urea compound	Pesticide
Meat Room Degreaser (#3)	-	Formulation	Degreaser
2-Mercaptopyrimidine	1450-85-7	Acyl halide	Anti-infective; Anti-fungal; Preservative
Metal Cleaner (#20)	-	Formulation	Cleaner
Methanol	67-56-1	Alcohol	Solvent
2-Methoxyethanol	109-86-4	Alcohol	Solvent; Plasticizer
Methyl acetate	79-20-9	Ester	Solvent; Chemical intermediate; Synthetic flavor ingredient
Methyl cyanoacetate	105-34-0	Ester, Nitrile compound	Adhesive; Pharmaceutical intermediate

Chemical and Product Classes of Substances Tested in the BCOP Assay

Substance	CASRN[1]	Chemical Class	Product Class
Methyl cyclopentadiene dimer	-	Cyclic hydrocarbon	Solvent/industrial chemical; Petrochemical product
Methylcyclopentane	96-37-7	Ketone	Solvent; Manufacture of lacquers, varnishes, cosmetics, pharmaceuticals
Methyl ethyl ketone	78-93-3	Ketone	Solvent; Manufacture of lacquers, varnishes, cosmetics, pharmaceuticals
Methyl isobutyl ketone	108-10-1	Ketone	Solvent; Synthetic flavor; Drycleaning
1-Methylpropyl benzene	135-98-8	Hydrocarbon (cyclic)	Not classified
Mild Shampoo (HZJ) 25%	-	Ketone	Solvent; Synthetic flavor; Drycleaning
MYRJ-45	-	Ketone	Solvent; Manufacture of lacquers, varnishes, cosmetics, pharmaceuticals
1-Naphthalene acetic acid	86-87-3	Carboxylic acid, Polycyclic compound	Pesticide
1-Naphthalene acetic acid, Na salt	61-31-4	Carboxylic acid (salt), Polycyclic compound	Pesticide
1-Nitropropane	108-03-2	Hydrocarbon (acyclic), Nitro compound	Solvent, Chemical intermediate
n-Octanol	111-87-5	Alcohol	Solvent; Fragrance
Parafluoraniline	371-40-4	Amine/Amidine	Intermediate for herbicides; Dyes
2,4-Pentanedione	123-54-6	Ketone	Solvent; Plasticizer
Petroleum ether	8032-32-4	Hydrocarbon (acyclic)	Solvent
Petroleum wax	-	Wax	Petrochemical product
Phenylbutazone	50-33-9	Heterocyclic	Pharmaceutical
1-Phenyl-3-pyrazolidone	92-43-3	Heterocyclic	Photographic agent
Polishing Scrub (HZT) 100%	-	Formulation	Surfactant-containing formulation
Polyalkenylsuccinate ester/amine salt	-	Amidine	Lubricant additive; Petrochemical product
Polyethylene glycol 400	25322-68-3	Alcohol, Polyether	Surfactant (nonionic), Lubricant, Plasticizer, Solvent

Chemical and Product Classes of Substances Tested in the BCOP Assay

Substance	CASRN[1]	Chemical Class	Product Class
Polyethylene glycol 600	-	Alcohol, Polyether	Surfactant (nonionic)
Pot and Pan Cleaner (#8)	-	Formulation	Cleaner
Potassium cyanate	590-28-3	Inorganic salt	Herbicide; Pharmaceutical intermdiate
Process oil	-	Oil	Petrochemical product
Promethazine hydrochloride	58-33-3	Amine/Amidine, Heterocyclic, Organic sulfur compound	Antihistamine; Anti-nausea drug
Propylene glycol	57-55-6	Alcohol	Solvent
Propyl-4-hydroxybenzoate	94-13-3	Carboxylic acid, Phenol	Antimicrobial
Pyridine	110-86-1	Heterocyclic	Solvent; Intermediate for pharmaceuticals, dyes, pesticides
Quinacrine	69-05-6	Heterocyclic	Drug/Therapeutic agent
Shampoo No. 1 (HZC) 25%	-	Formulation	Surfactant-containing formulation
Shampoo No. 2 (HZX)	-	Formulation	Surfactant-containing formulation
Shampoo No. 3 (HZM) 25%	-	Formulation	Surfactant-containing formulation
Shampoo No. 4 (HZV) 25%	-	Formulation	Surfactant-containing formulation
Shampoo No. 5 (HZD) 25%	-	Formulation	Surfactant-containing formulation
Shampoo No. 6 (HZN) 25%	-	Formulation	Surfactant-containing formulation
Shampoo No. 7 (HZA)	-	Formulation	Surfactant-containing formulation
Shampoo No. 8 (HZG) 25%	-	Formulation	Surfactant-containing formulation
Shower Gel (HZS) 100%	-	Formulation	Surfactant-containing formulation
Skin Cleanser (HZI) 100%	-	Formulation	Surfactant-containing formulation
Sodium hydroxide (1%)	1310-73-2	Alkali	Caustic agent

Chemical and Product Classes of Substances Tested in the BCOP Assay

Substance	CASRN[1]	Chemical Class	Product Class
Sodium hydroxide (10%)	1310-73-2	Alkali	Caustic agent
Sodium lauryl sulfate (15 %)	151-21-3	Carboxylic acid (salt)	Surfactant (anionic); Detergent
Sodium lauryl sulfate (3 %)	151-21-3	Carboxylic acid (salt)	Surfactant (anionic); Detergent
Sodium lauryl sulfate (30 %)	151-21-3	Carboxylic acid (salt)	Surfactant (anionic); Detergent
Sodium oxalate	62-76-0	Carboxylic acid (salt)	Textile finishing; Pyrotechnic, Industrial byproduct
Sodium perborate	10486-00-7	Inorganic salt, Boron compound	Household cleaner; Detergent
Tetraaminopyrimidine sulfate	5392-28-9	Amine, Heterocycle, Inorganic salt	Not classified
Thiadiazole alkyl derivative	-		Lubricant additive; Petrochemical product
Thiourea	62-56-6	Organic sulfur compound	Photographic agent; Flame-retardant; Chelation reagent and catalyst; Chemical intermediate;
Toilet Bowl Cleaner (#1)	-	Formulation	Cleaner
Toilet Bowl Cleaner (#4)	-	Formulation	Cleaner
Toluene	108-88-3	Hydrocarbon (cyclic)	Solvent
Trichloroacetic acid (3%)	76-03-9	Carboxylic acid	Caustic agent; Fixative; Herbicide
Trichloroacetic acid (30%)	76-03-9	Carboxylic acid	Caustic agent; Fixative; Herbicide
1,2,3-Trichloropropane	96-18-4	Hydrocarbon (halogenated)	Solvent
Triethanolamine	102-71-6	Amine, Alcohol	Antimicrobial, Chemical intermdiate
1,2,4-Trimethylbenzene	95-63-6	Hydrocarbon (cyclic)	Chemical intermediate
Triton X-100 (1%)	9002-93-1	Ether	Surfactant (nonionic)
Triton X-100 (10 %)	9002-93-1	Ether	Surfactant (nonionic), Detergent, Emulsifier
Triton X-100 (5%)	9002-93-1	Ether	Surfactant (nonionic)

Chemical and Product Classes of Substances Tested in the BCOP Assay

Substance	CASRN[1]	Chemical Class	Product Class
Triton X-155	9010-44-0	Ether	Surfactant (nonionic)
Tween 20	9005-64-5	Ester, Polyether	Surfactant (nonionic); Detergent
Xylene	1330-20-7	Hydrocarbon (cyclic)	Agricultural chemical

[1] CASRN = Chemical Abstracts Service Registry Number

This page intentionally left blank

Annex I-2

Components of Formulations Tested in Gettings et al. (1996)

This page intentionally left blank

Components of Formulations Tested in Gettings et al. (1996)

Formulation	Formulation Components	% (W/W)
HZA-Shampoo No. 7	Water	53.86
	Sodium lauryl sulfate (30%)	25.00
	Disodium laureth sulfocuccinate (40%)	15.00
	Lauramide DEA	0.50
	Butylene glycol	5.00
	Methyl and propylparabens	0.25
	Carageenan	0.35
	Methyl and methylchloroisothiazolinone	0.04
HZB-Liquid Soap No. 1	Water and volatiles	65-85
	Ammonium lauryl sulfate	1-10
	Sodium laureth sulfate	1-10
	Lauramide DEA	1-10
	Glycerine	1-10
	Isostearamidopropyl morpholine lactate	0.1-1.0
	Disodium ricinoleamido MEA-sulfosuccinate	0.1-1.0
	DMDM hydantoin	0.1-1.0
	Citric acid	0.1-1.0
	Triclosan	0.1-1.0
	Tetrasodium EDTA	<0.1
	FD&C Yellow No. 5	<0.1
	FD&C Red No. 4	<0.1
HZC-Shampoo No. 1	Water	14.037
	Laurylamidopropyl betaine (30%)	60.000
	Cetrimonium chloride	16.000
	PEG-3 cocamide	4.500
	Citric acid	3.500
	Sodium chloride	1.000
	Ditallowdimonium chloride (73%)	0.700
	Lauryl alcohol	0.250
	Methyl and chloroisothiazolinone (1.5%)	0.033

Components of Formulations Tested in Gettings et al. (1996)

Formulation	Formulation Components	% (W/W)
HZD-Shampoo No. 5	Water	54.120
	Sodium laureth sulfate (26%)	38.00
	Cocamide DEA	3.000
	Cocamide propyl betaine (37%)	1.750
	Disodium EDTA	0.050
	Methylparaben	0.150
	Propylparaben	0.100
	Citric acid	0.250
	FD&C Yellow No. 5 (1%)	0.050
	D&C Red No. 33 (0.5%)	0.015
	DMDM hydantoin (54%)	0.300
	BHT	0.050
	Sodium glutamate	2.000
	Sodium chloride	0.170
HZE-Gel Cleanser	Water	59.974
	Acylglutamate CT-12 (30%)	15.000
	Cocoamphodiacetate (50%)	15.000
	Sodium nonoxynol-6 phosphate (88.5%)	6.000
	Quaternium-26 (58%)	1.500
	PEG-120-methyl glucose dioleate	1.500
	Citric acid	0.100
	Sodium citrate	0.500
	Disodium EDTA	0.050
	Methylparaben	0.150
	DMDM hydentoin (55%)	0.200
	FD&C Yellow No. 10 (1%)	0.001
	D&C Blue No. 1 (0.746%)	0.025
HZF-Baby Shampoo No. 2	Water	57.653
	Sodium laureth (2EO) sulfate (28%)	21.430
	Disodium laureth-3-sulfosuccinate (40%)	9.090
	Cocamidopropyl betaine (30%)	10.000
	Lauramide DEA	1.500
	Kathon CG (1.5%)	0.067
	Tetrasodium EDTA (30%)	0.260

Components of Formulations Tested in Gettings et al. (1996)

Formulation	Formulation Components	% (W/W)
HZG-Shampoo No. 8	Water	48.43
	Sodium laureth sulfate (28%)	20.00
	Sodium lauryl sulfate (30%)	25.00
	Lauramide-DEA	5.00
	Hydroxyethyl tallow glycinate	1.00
	Citric acid	0.20
	PEG-45M	0.20
	Methyl and propylparabens	0.13
	Methyl and chloromethyl-isothiazolinone	0.04
HZH-Eye Make-Up Remover	Water	96.242
	Sodium laureth sulfate (21%)	0.900
	Cocoamphocarboxyglycinate (40%)	1.100
	Hexylene glycol	1.000
	Dipotassium phosphate	0.394
	Potassium phosphate	0.102
	Allantoin	0.050
	Methyl paraben	0.150
	EDTA	0.150
	Rose water	0.008
	Thimerosal	0.003
HZI-Skin Cleanser	Water	44.0
	Sodium laureth sulfate (30%)	50.0
	Cocamide MEA	5.0
	Sodium chloride	0.4
	Disodium EDTA	0.2
	Imidizolidinyl urea	0.2
	Methylparaben	0.2
	Benzoic acid	0.1
HZJ-Mild Shampoo	Water	52.09
	Tween 20	12.63
	Cocoamphodiacetate (24%)	21.25
	PEG 6000	2.60
	Cedepal TD403 (75%)	6.53
	Hydrochloric acid (15%)	1.68
	Arlacel 20	0.92
	Benzyl alcohol	0.10
	Dowicil 200	0.10
	D&C Yellow No. 10 (0.2%)	1.70
	D&C Orange No. 4 (0.2%)	0.20

Components of Formulations Tested in Gettings et al. (1996)

Formulation	Formulation Components	% (W/W)
HZK-Bubble Bath	Water	68.75
	Sodium laureth sulfate (60%)	25.00
	Lauramide DEA	4.50
	SD Alcohol 3-A	3.75
	Sodium chloride	0.80
	Triethanolamine	0.40
	Phosphoric acid (86.5%)	0.35
	Sorbic acid	0.20
HZL-Foam Bath	Water	47.760
	Sodium laureth sulfate (26%)	46.000
	Cocamido propyl betaine (30%)	2.500
	Sodium chloride	2.400
	Glycol monostearate	0.400
	Color solution	0.300
	DMDM hydantoin (54%)	0.250
	Methylparaben	0.200
	Propylparaben	0.100
	BHT	0.050
	Aloe vera gel	0.015
	Citric acid	0.016
	Tetrasodium EDTA	0.010
HZM-Shampoo No. 3	Water	80-90
	Ammonium lauryl sulfate	5-10
	Lauramide DEA	1-5
	Cocamidopropyl sultaine	1-5
	Citric acid	<1.0
	Ammonium chloride	<1.0
	DMDM Hydantoin	<1.0
	Tetrasodium EDTA	<1.0
	Methylparaben	<1.0
	FD&C Yellow No. 5	<0.1
	D&C Yellow No. 10	<0.1
	FD&C Red No. 4	<0.1
	PPG-9	
HZN-Shampoo No. 6	Water	44.381
	Sodium laureth (2EO) sulfate (28%)	43.634
	Cocamidopropyl betaine (30%)	11.760
	Tetrasodium EDTA	0.125
	Formalin	0.100

Components of Formulations Tested in Gettings et al. (1996)

Formulation	Formulation Components	% (W/W)
HZP-Baby Shampoo No. 1	Water	49.54
	PEG-80 sorbitan laurate (50%)	23.60
	Sodium trideceth sulfate (50%)	17.40
	Lauroamphocarboxyglycinate (50%)	5.40
	PEG-150 distearate (50%)	5.00
	Cocamidoroyl hydroxysultane (50%)	4.00
	Sodium laureth-13 carboxylate (50%)	1.00
	Quaternium 15	0.03
	Benzyl alcohol	0.05
	FD&C Yellow No. 5 (1.0%)	0.25
	FD&C Yellow No. 6 (1.0%)	0.05
	Citric acid	0.08
HZQ-Cleansing Gel	Water	68.93
	Lauramphocarboxyglycinate (25%)	10.40
	Sodium trideceth sulfate (16%)	10.60
	TEA-lauryl sulfate (40%)	3.50
	Lauramide DEA	0.50
	PEG-150 distearate	2.80
	Propylene glycol	1.40
	Hexylene glycol	1.05
	Citric acid	0.28
	Diazolidinyl urea	0.20
	Methylparaben	0.20
	Sodium citrate	0.14

Components of Formulations Tested in Gettings et al. (1996)

Formulation	Formulation Components	% (W/W)
HZR-Facial Cleansing Foam	Water	32.97
	Sodium cocoyl isethionate	20.00
	Sodium lauroyl sarcosinate (30%)	25.00
	PPG-5-ceteth-10 phosphate	4.00
	Linoleamide DEA	2.00
	Sorbitol (70%)	2.75
	Glycol stearate	5.50
	Glycerin	2.00
	Diglycerol	2.00
	Cetearyl alcohol	2.75
	Mineral oil	0.50
	Methylparaben	0.15
	Propylparaben	0.10
	Trisodium EDTA	0.10
	Beeswax	0.10
	Ceresin	0.06
	Sodium borate	0.02
HZS-Shower Gel	Water	27.567
	Sodium lauroyl sarcosinate (30%)	25.000
	Laurimidopropyl betaine (30%)	25.000
	Cocamidopropyl hydroxysultaine (50%)	15.000
	Linoleamide DEA	4.500
	Glycol stearate	1.000
	Polyquaternium-2	1.000
	Phosphoric acid (86.5%)	0.600
	Tetrasodium EDTA	0.200
	BHT	0.050
	PPG-12-buteth-16	0.050
	Methyl and chlorosothiazolinone (1.5%)	0.033

Components of Formulations Tested in Gettings et al. (1996)

Formulation	Formulation Components	% (W/W)
HZT-Polishing Scrub	Water	33.85
	Mineral oil	10.00
	Lauroamphocarboxyglycinate (25%)	8.80
	Sodium trideceth sulfate (16%)	9.40
	Petrolatum	6.60
	Isopropyl palmitate	6.60
	Propylene glycol	5.00
	Cetyl palmitate	4.40
	Glyceryl stearate and PEG-100 stearate	4.40
	Aluminum silicate	3.00
	Cetyl alcohol	2.50
	Polypropylene	2.50
	Magnesium aluminum silicate	1.00
	Titanium dioxide	0.50
	Hexylene glycol	0.40
	Imidazolidinyl urea	0.30
	Methylparaben	0.30
	Lactic acid	0.25
	Propylparaben	0.20
HZU-Hand Soap	Water	37.95
	Sodium C14-16 olefin sulfonate (36%)	20.25
	Sodium lauroyl sarcosinate	20.00
	Cocamidopropyl hydroxysultaine	8.00
	Propylene glycol	3.00
	Glycerol stearate	3.00
	PPG-12-PEG-50 lanolin	3.00
	Polyquaternium-7	2.00
	Citric acid	1.00
	Hydrolysed animal protein	1.00
	Polyquaternium-10	0.50
	Quaternium-15	0.20
	Aloe vera gel	0.10

Components of Formulations Tested in Gettings et al. (1996)

Formulation	Formulation Components	% (W/W)
HZV-Shampoo No. 4	Water	80-90
	Ammonium lauryl sulfate	5-10
	Lauramide DEA	1-5
	Cocamidopropyl sultaine	<1.0
	Ammonium chloride USP	<1.0
	Citric acid	<1.0
	DMDM hydantoin	<1.0
	Tetrasodium EDTA	<1.0
	Methylparaben	<1.0
	FD&C Yellow No. 5	<1.0
	D&C Yellow No. 10	<1.0
	FD&C Red No. 4	<1.0
HZW-Liquid Soap No. 2	Water and volatiles	60-80
	TEA-lauryl sulfate	1-10
	Sodium laureth sulfate	1-10
	Sodium lauroyl sarcosinate	1-10
	Lauramide DEA	1-10
	Glycol distearate	1-10
	Isostearamideopropyl morpholine lactate	0.1-1.0
	Disodium ricinoleamido MEA-sulfosuccinate	0.1-1.0
	DMDM hydantoin	0.1-1.0
	Citric acid	0.1-1.0
	Tetrasodium EDTA	<0.1
HZX-Shampoo No. 2	Water	69.1895
	Ammonium lauryl sulfate (25%)	25.0000
	Cocamide DEA	3.0000
	Hydroxypropyl methylcellulose	1.4500
	EDTA	0.6000
	Formaldehyde	0.2000
	Benzyl alcohol	0.2000
	Benzophenone-4 sodium hydroxide	0.0400
	Citric acid	0.0100
	Ammonium chloride	0.0100
	FD&C Blue No. 1	0.0005

Components of Formulations Tested in Gettings et al. (1996)

Formulation	Formulation Components	% (W/W)
HZY-Anti-Dandruff Shampoo	Water	27.13
	Sodium lauroyl sarcosinate (30%)	15.00
	Lauramide DEA	4.50
	TEA-lauryl sulfate (40%)	45.00
	Glycol distearate	3.00
	Zinc pyrithione	2.10
	Sodium chloride	1.20
	Citric acid	0.90
	Imidazolidinyl urea	0.50
	Methylparaben	0.30
	Propylparaben	0.10
	Xanthan gum	0.27
HZZ-Facial Cleanser	Water	32.55
	Mineral oil	40.00
	Beeswax	2.30
	PEG-16 soya sterol	5.00
	PEG-8 dilaurate	2.00
	Cetearyl alcohol (70%)	0.80
	Ceteareth 20 (30%)	0.80
	Beheme acid	0.80
	Sodium borate	0.75
	Ceresin	0.50
	Carbopol dispersion (25%)	15.00
	Methylparaben	0.15
	Propylparaben	0.10
	Disodium EDTA	0.05

This page intentionally left blank

Annex I-3

Components of Formulations Tested in Swanson et al. (1995)

This page intentionally left blank

Components of Formulations Tested in Swanson et al. (1995)

Code	Formulation	Formulation Components	Percentage	pH
1	Toilet Bowl Cleaner	Water	90-95	8.8
		Nonionic Surfactant	1-5	
		Anionic Surfactant	1-5	
		Preservative	< 1	
		Thickener	< 1	
		Dye	< 1	
		Fragrance	< 1	
		Phosphate	< 1	
2	Floor Cleaner	Water	90-95	10.8
		Anionic Surfactant	1-5	
		Nonionic Surfactant	1-5	
		MEA	< 1	
		Fragrance	< 1	
		Dye	< 1	
3	Meat Room Degreaser	Water	80-85	12.65
		Anionic surfactant	1-5	
		Nonionic surfactant	1-5	
		Chelator	1-5	
		Glycol ether	1-5	
		Inorganic salt	1-5	
		KOH	< 1	
4	Toilet Bowl Cleaner	Water	90-95	2.5
		Organic acid	1-5	
		Anionic surfactant	1-5	
		Thickener	< 1	
		Dye	< 1	
		Fragrance	< 1	
5	All Purpose Cleaner	Water	90-95	13
		Nonionic surfactant	1-5	
		Inorganic salt	1-5	
		NaOH	1-5	
		Chelator	< 1	
		KOH	< 1	
		Anionic surfactant	< 1	
		Fragrance	< 1	
		Dye	< 1	
6	Bathroom Cleaner	Water	80-85	13
		Chelator	10-15	
		Glycol ether	1-5	
		Nonionic surfactant	1-5	
		Quaternary compound	< 1	

Components of Formulations Tested in Swanson et al. (1995)

Code	Formulation	Formulation Components	Percentage	pH
7	All Purpose Cleaner	Water	80-85	14
		Inorganic salt	5-10	
		NaOH	1-5	
		Nonionic surfactant	1-5	
		Anionic surfactant	1-5	
		KOH	< 1	
		Chelator	< 1	
		Amphoteric surfactant	< 1	
		Fragrance	< 1	
		Dye	< 1	
8	Pot and pan cleaner	Water	60-65	7.8
		Anionic surfactant	25-30	
		Nonionic surfactant	5-10	
		Glycol ether	1-5	
		Preservative	< 1	
		Dye	< 1	
9	Heavy-duty cleaner/degreaser	Water	75-80	13.6
		Inorganic salts	10-15	
		Chelator	1-5	
		NaOH	1-5	
		Nonionic surfactant	1-5	
		Amphoteric surfactant	1-5	
		Dye	< 1	
10	Floor cleaner	Water	85-90	11.7
		MEA	1-5	
		Anionic surfactant	1-5	
		Glycol ether	1-5	
		Ammonium hydroxide	1-5	
		Nonionic surfactant	< 1	
		Chelator	< 1	
		Fragrance	< 1	
		Dye	< 1	
11	General Cleaner	Water	70-75	1
		Inorganic acid	15-20	
		Nonionic surfactant	1-5	
		Amphoteric surfactant	< 1	
		Fragrance	< 1	
		Dye	< 1	
12	General Cleaner	Water	75-80	14
		Anionic surfactant	10-15	
		Nonionic surfactant	5-10	
		Chelator	1-5	
		Inorganic salt	1-5	

Components of Formulations Tested in Swanson et al. (1995)

Code	Formulation	Formulation Components	Percentage	pH
13	Cleaner/Degreaser	Water	65-70	12
		Glycol ether	10-15	
		Anionic surfactant	1-5	
		Inorganic salt	1-5	
		Chelator	1-5	
		Nonionic surfactant	5-10	
		NaOH	1-5	
		Dye	< 1	
14	Floor stripper	Water	50-55	11.5
		Glycol ether	30-35	
		MEA	10-15	
		Organic solvent	1-5	
		Ammonium hydroxide	1-5	
		Anionic surfactant	1-5	
15	Heavy Duty cleaner	Water	65-70	13.5
		Inorganic salts	10-15	
		Anionic surfactant	5-10	
		Chelator	1-5	
		NaOH	1-5	
		Nonionic surfactant	1-5	
		Amphoteric surfactant	1-5	
16	Degreaser	Water	65-70	12.9
		Anionic surfactant	5-10	
		Chelator	5-10	
		Nonionic surfactant	5-10	
		KOH	1-5	
		Inorganic salt	1-5	
		Glycol ether	1-5	
17	Floor stripper	Water	60-65	13.1
		Glycol ether	10-15	
		Anionic surfactant	10-15	
		MEA	5-10	
		Organic solvent	1-5	
		Inorganic salt	1-5	
		NaOH	1-5	
		Chelator	< 1	
		Flurochemical	< 1	
		Fragrance	< 1	

Components of Formulations Tested in Swanson et al. (1995)

Code	Formulation	Formulation Components	Percentage	pH
18	Floor stripper	Water	55-60	14
		Glycol ether	10-15	
		Inorganic salt	5-10	
		Amphoteric surfactant	5-10	
		MEA	1-5	
		NaOH	1-5	
		Chelator	1-5	
		Nonionic surfactants	< 1	
		Fragrance	< 1	
19	Glass cleaner	Water	65-70	12.1
		Glycol ether	20-25	
		Ammonium hydroxide	1-5	
		Anionic surfactant	1-5	
		Chelator	1-5	
		Dye	< 1	
20	Metal cleaner	Water	65-70	14
		Chelator	5-10	
		NaOH	5-10	
		Nonionic surfactant	5-10	
		KOH	< 1	

Annex I-4

Components of Formulations Tested in Swanson and Harbell (2000)

This page intentionally left blank

Components of Formulations Tested in Swanson and Harbell (2000)

Substance	Group	Formulation Component	Percentage
1	1-1	Cyclomethicone	50-55
		Alcohol	40-45
		Active	10-15
2	1-2	Dimethicone (alkoxylated derivative)	50-55
		Alcohol	40-45
		Active	10-15
3	1-3	Alcohol	40-45
		Cyclomethicone	30-35
		Dimethicone (alkoxylated derivative)	20-25
		Active	10-15
4	2-4	Isoparaffinic hydrocarbon	80-85
		Active	10-15
		Cyclic polysiloxane	5-10
		Emollient	< 1
5	2-5	Isoparaffinic hydrocarbon	80-85
		Active	10-15
		Cyclic polysiloxane	5-10
		Alcohol	1-5
		Emollient	< 1
6	2-6	Isoparaffinic hydrocarbon	75-80
		Active	10-15
		Cyclic polysiloxane	5-10
		Alcohol	5-10
		Emollient	< 1
7	2-7	Isoparaffinic hydrocarbon	70-75
		Active	10-15
		Alcohol	10-15
		Cyclic polysiloxane	5-10
		Emollient	< 1
8	2-8	Isoparaffinic hydrocarbon	65-70
		Alcohol	15-20
		Active	10-15
		Cyclic polysiloxane	5-10
		Emollient	< 1
9	3-9	Alcohol	60-65
		Water	25-30
		Active	10-15
		Fragrance	< 1
10	3-10	Water	45-50
		Alcohol	40-45
		Active	10-15
		Fragrance	< 1
11	3-11	Water	55-60
		Alcohol	30-35
		Active	10-15
		Fragrance	< 1
12, 13	Benchmark	Alcohol	85-90
		Active	10-15
		Dimethicone	1-5
		Fragrance	< 1
14, 15	Ethanol	Ethanol	100
16	Vehicle control	Alcohol	85-90
		Water	10-15
		Dimethicone	1-5
		Fragrance	< 1

This page intentionally left blank

Annex I-5

Components of AMCP Formulations Tested

This page intentionally left blank

Components of AMCP Formulations Tested

Formulation Name	Formulation Components	Percentage
A, Disinfectant/Cleaner Concentrate	Water	65-70
	Inorganic Salt	1-5
	Inorganic Phosphate	1-5
	Quaternary	1-5
	Nonionic Surfactant	1-5
	pH adjuster	<1
	Chelator	<1
	Fragrance	<1
	Dyes	<1
B, Disinfectant/Cleaner Concentrate	Water	60-65
	Fragrance	<1
	Dye	<1
	Quaternary	15-20
	Chelator	1-5
	Inorganic Salt	1-5
	Nonionic Surfactant	1-5
C, Disinfectant/Cleaner Concentrate	Water	75-80
	Hydrogen Peroxide	5-10
	Solvent	1-5
	Anionic Surfactant	1-5
	Inorganic Alkalis	1-5
	Organic Acid	1-5
	Corrosion Inhibitors	1-5
	Organic Acid	1-5
	Nonionic Surfactant	<1
	Inorganic Salt	<1
	Inorganic Salt	<1
	Inorganic Acid	<1
	Organic Acid	<1
	Organic Acid	<1
	Organic Acid	1-5
D, Stone Floor Polish	Water	60-65
	Organic Acid	30-35
	Thickeners	5-10
	Plasticizers	<1
E, Disinfectant/Cleaner Concentrate	Water	75-80
	Ouaternary	1-5
	Inorganic Phosphates	1-5
	Inorganic Salt	1-5
	Nonionic Surfactant	1-5
	pH adjuster	<1
	Chelator	<1
	Fragrance	<1
	Dye	<1
F, Disinfectant/Cleaner Concentrate	Water	60-65
	Inorganic Acid	1-5
	Organic Acid	5-10
	Anionic Surfactant	1-5
	Organic Acid	<1
	Corrosion Inhibitor	<1
	Nonionic Surfactant	1-5
	Solvent	5-10
	Fragrance	<1

Components of AMCP Formulations Tested

Formulation Name	Formulation Components	Percentage
	Hydrogen Peroxide	1-5
G, Disinfectant/Cleaner Concentrate	Water	40-50
	Solvent	25-35
	Chelator	1-5
	Amine	5-10
	Quaternary	1-5
	Nonionic Surfactant	10-15
	Fragrance	1-5
	Dyes	<1
H, Disinfectant/Cleaner Concentrate	Water	90-95
	Solvent	1-5
	Inorganic Phosphates	1-5
	Nonionic Surfactant	<1
	Inorganic Alkalis	<1
	Quaternary	<1
	Inorganic Salt	<1
	Fragrance	<1
	Nonionic Surfactant	<1
	Thickeners	<1
I, Disinfectant/Cleaner RTU	Water	95-100
	Inorganic Salt	<1
	Inorganic Phosphate	<1
	Quaternary	<1
	Nonionic Surfactant	<1
	pH adjuster	<1
	Chelator	<1
	Fragrance	<1
	Dyes	<1
J, Disinfectant/Cleaner RTU	Water	90-95
	Solvent	1-5
	Nonionic Surfactant	1-5
	pH adjuster	<1
	Fragrance	<1
	Quaternary	<1
	Amine	<1
	Thickeners	<1
	Dye	<1
K, Disinfectant	Water	95-100
	Inorganic Salt	<1
	Anionic Surfactant	<1
	TCM	<1
	pH adjuster	<1
L, Disinfectant/Cleaner RTU	Water	95-100
	Fragrance	<1
	Dye	<1
	Quaternary	<1
	Chelator	<1
	Inorganic Salt	<1
	Nonionic Surfactant	<1

Components of AMCP Formulations Tested

Formulation Name	Formulation Components	Percentage
M, Disinfectant/Cleaner RTU	Water	85-90
	Solvent	5-10
	Chelator	1-5
	Nonionic Alkoxylate	<1
	Quaternary	<1
	Inorganic Salt	<1
	Fragrance	<1
N, Disinfectant/Cleaner Concentrate	Water	70-75
	Nonionic Surfactant	5-10
	Nonionic Surfactant	5-10
	Hydrotrope	5-10
	Chelator	<1
	pH adjuster	<1
	Hydrogen Peroxide	1-5
O, Disinfectant/Cleaner Concentrate	Water	95-100
	Quaternary	<1
	Nonionic Alkoxylate	<1
	Chelator	<1
	Inorganic Salt	<1
	Fragrance	<1
	Dye	<1
P, Disinfectant/Cleaner RTU	Water	95-100
	Phenolics	<1
	Anionic Surfactant	<1
	Solvent	<1
	Chelator	<1
	pH adjuster	<1
	Solvent	<1
	Anionic Surfactant	<1
	Antioxidants	<1
	Inorganic Salts	<1
	Fragrance	<1
	Dye	<1
Q, Disinfectant/Cleaner	Water	95-100
	Nonionic Surfactant	<1
	Fragrance	<1
	Solvent	<1
	pH adjuster	<1
	Quaternary	<1
R, Disinfectant	Water	95-100
	Quaternary	<1
	Dye	<1
S, Acid Toilet Bowl Cleaner	Water	90-95
	Inorganic Acid	5-10
	Anionic Surfactant	1-5
	Inorganic Salts	<1
	Dye	<1
T, Descaler	Water	75-80
	Inorganic Acid	10/15
	pH adjuster	1-5

Components of AMCP Formulations Tested

Formulation Name	Formulation Components	Percentage
U, Disinfectant/Cleaner RTU	Water	95-100
	Quaternary	<1
	Inorganic Phosphates	<1
	Inorganic Salt	<1
	Nonionic Surfactant	<1
	pH adjuster	<1
	Chelator	<1
	Fragrance	<1
	Dye	<1
V, Disinfectant/Cleaner RTU	Water	95-100
	Solvent	1-5
	Chelator	<1
	Amine	<1
	Quaternary	<1
	Nonionic Surfactant	<1
	Fragrance	<1
	Dyes	<1
W, Disinfectant RTU	Water	95-100
	Quaternary	<1
	Fragrance	<1
AB (active ingredient)	Alkyl (60% C14, 30% C16, 5% C12, 5% C18) Dimethyl Benzyl Ammonium Chloride	3%
AF, Disinfectant (active ingredient)	Octanoic Acid	0.138%
AG, Metal Cleaner	Water	65-70
	Chelator	5-10
	NaOH	5-10
	Nonionic Surfactant	5-10
	KOH	<1
AH, Degreaser	Water	65-70
	Anionic Surfactant	5-10
	Chelator	5-10
	Nonionic Surfactant	5-10
	KOH	1-5
	Inorganic Salt	1-5
	Glycol Ether	1-5
AI, Heavy Duty Cleaner/Degreaser	Water	75-80
	Inorganic Salts	10-15
	Chelator	1-5
	NaOH	1-5
	Nonionic Surfactant	1-5
	Amphoteric Surfactant	1-5
	Dye	<1
AJ, Heavy Duty Cleaner	Water	65-70
	Inorganic Salts	10-15
	Anionic Surfactant	5-10
	Chelator	1-5
	NaOH	1-5
	Nonionic Surfactant	1-5
	Amphoteric Surfactant	1-5

Components of AMCP Formulations Tested

Formulation Name	Formulation Components	Percentage
AK, Floor Stripper	Water	55-60
	Glycol Ether	10-15
	Inorganic Salt	5-10
	Amphoteric Surfactant	5-10
	MEA	1-5
	NaOH	1-5
	Chelator	1-5
	Nonionic Surfactants	<1
	Fragrance	<1
AL, Cleaner/Degreaser	Water	65-70
	Glycol Ether	10/15
	Anionic Surfactant	1-5
	Inorganic Salt	1-5
	Chelator	1-5
	Nonionic Surfactant	5-10
	NaOH	1-5
	Dye	<1
AM, Glass Cleaner	Water	65-70
	Glycol Ether	20-25
	Ammonium Hydroxide	1-5
	Anionic Surfactant	1-5
	Chelator	1-5
	Dye	<1
AN, General Cleaner	Water	75-80
	Anionic Surfactant	10-15
	Nonionic Surfactant	5-10
	Chelator	1-5
	Inorganic Salt	1-5
AO, Floor Stripper	Water	60-65
	Glycol Ether	10-15
	Anionic Surfactant	10-15
	MEA	5-10
	Organic Solvent	1-5
	Inorganic Salt	1-5
	NaOH	1-5
	Chelator	<1
	Flurochemical	<1
	Fragrance	<1
AP, All Purpose Cleaner	Water	80-85
	Inorganic Salt	5-10
	NaOH	1-5
	Nonionic Surfactant	1-5
	Anionic Surfactant	1-5
	KOH	<1
	Chelator	<1
	Amphoteric Surfactant	<1
	Fragrance	<1
	Dye	<1
AQ, Drain Cleaner	Water	55-60
	Bleach	5-10
	Nonionic Surfactant	5-10
	Inorganic Base	<1
	Inorganic Salt	<1

Components of AMCP Formulations Tested

Formulation Name	Formulation Components	Percentage
AR, Drain Cleaner	Water	55-60
	Bleach	5-10
	Nonionic Surfactant	5-10
	Inorganic Base	1-5
	Inorganic Salt	<1
AS, Drain Cleaner	Water	40-45
	Inorganic Salt	5-10
	Nonionic Surfactant	5-10
	Inorganic Alkalis	<1
	Inorganic Salt	<1
AT, Drain Cleaner	Water	55-60
	Bleach	5-10
	Inorganic Base	<1
	Inorganic Salt	<1
	Anionic Surfactant	<1
AU, Drain Cleaner	Water	55-60
	Bleach	5-10
	Inorganic Base	1-5
	Inorganic Salt	<1
	Anionic Surfactant	<1
AV, Drain Cleaner	Water	55-60
	Bleach	5-10
	Inorganic Base	1-5
	Inorganic Salt	<1
	Anionic Surfactant	<1
AW, Drain Cleaner	Water	70-75
	Bleach	1-5
	Nonionic Surfactant	<1
	Inorganic Base	<1
	Inorganic Salt	<1
AX, Floor Stripper	Water	50-55
	Glycol Ether	30-35
	MEA	10-15
	Organic Solvent	1-5
	Ammonium Hydroxide	1-5
	Anionic Surfactant	1-5
AY, Drain Cleaner	Water	65-70
	Bleach	25-30
	Inorganic Salt	1-5
	Inorganic Base	1-5
	Anionic Surfactant	<1
	Defoamer	<1
BB, Glass Cleaner	Water	90-95
	Dye	<1
	Inorganic Base	<1
	Chelator	<1
	Solvent	5-10

Components of AMCP Formulations Tested

Formulation Name	Formulation Components	Percentage
BD, Heavy-duty Cleaner/Degreaser	Water	75-80
	Inorganic Salts	10-15
	Chelator	1-5
	NaOH	1-5
	Nonionic Surfactant	1-5
	Amphoteric Surfactant	1-5
	Dye	<1
BE, Toilet Bowl Cleaner	Water	90-95
	Organic Acid	1-5
	Anionic Surfactant	1-5
	Thickener	<1
	Dye	<1
	Fragrance	<1
BF, Floor Cleaner	Water	85-90
	MEA	1-5
	Anionic Surfactant	1-5
	Glycol Ether	1-5
	Ammonium Hydroxide	1-5
	Nonionic Surfactant	<1
	Chelator	<1
	Fragrance	<1
	Dye	<1
BJ, Bathroom Cleaner	Water	80-85
	Chelator	10-15
	Glycol Ether	1-5
	Nonionic Surfactant	1-5
	Quaternary Compound	<1
BK, Glass Cleaner	Water	90-95
	Dye	<1
	Solvent	1-5
	Anionic Surfactant	<1
	Fragrance	<1
	Chelator	<1
	Inorganic Base	<1
BL, Glass Cleaner	Water	90-95
	Chelator	<1
	Solvent	5-10
	Anionic Surfactant	<1
	Dye	<1
	Inorganic Base	<1
BM, Glass Cleaner	Water	90-95
	Solvent	5-10
	Inorganic Base	<1
	pH adjuster	<1
	Anionic Surfactant	<1
BN, Toilet Bowl Cleaner	Water	90-95
	Nonionic Surfactant	1-5
	Anionic Surfactant	1-5
	Preservative	<1
	Thickener	<1
	Dye	<1
	Fragrance	<1
	Phosphate	<1

Components of AMCP Formulations Tested

Formulation Name	Formulation Components	Percentage
BP, Glass Cleaner	Water	90-95
	Solvent	1-5
	Inorganic Base	<1
	Chelator	<1
	Dye	<1
	Anionic Surfactant	<1
	Fragrance	<1
BS	Water	50-74
	Sodium Tripolyphosphate	10-24
	Inorganic Salt	5-9
	Hydroxide	1-4
	Hydroxide	1-4
	Sodium Hypochlorite	1-4
	Thickener	1-4
	Minor Ingredients	<1
BQ, Glass Cleaner	Water	85-90
	Dye	<1
	Organic Acid	<1
	Inorganic Acid	<1
	Solvent	5-10
	Anionic Surfactant	<1
	Fragrance	<1
CG, Bathroom Cleaner	Water	65-70
	Solvent	5-10
	Propellant	5-10
	Surfactant	<1
	Chelator	15-20
	pH adjuster	<1
	Quaternary Compound	<1
CH, All Purpose Cleaner	Organic Acid	<1
	Water	90-95
	Solvent	1-5
	Fragrance	<1
	Dye	<1
	Surfactant	<1
CJ, Laundry Pre-Spotter	Inorganic Salt	55-60
	Enzyme	<1
	Dye	<1
	Silicones	5-10
	Inorganic Salt	25-30
	Nonionic Surfactant	1-5
CK	Surfactant	50-74
	Hydrocarbon	10-24
	Citric Acid (Anhydrous)	5-9
	Carbohydrate	1-4
	Hydroxide	1-4
	Water	1-4
	Perfume	1-4
	Minor Ingredients	<1

Components of AMCP Formulations Tested

Formulation Name	Formulation Components	Percentage
CN	Surfactant	25-49
	Silicone and Siloxanes	10-24
	Alkoxylated Alcohol	10-24
	Solvent	25-49
	Iach	10-24
	Perfume	1-4
	Minor Ingredients	<1
CO	Water	75-100
	Solvent	1-4
	Minor Ingredients	<1
CP	Water	25-49
	Polyol	10-24
	Surfactant	25-49
	Fatty Acid	5-9
	Solvent	5-9
	Hydroxide	1-4
	Aloe Vera Gel	1-4
	Minor Ingredients	<1
CQ	Water	50-74
	Surfactant	10-24
	Fatty Acid	10-24
	Polyols	5-9
	Hydroxide	1-4
	Ether	1-4
	Minor Ingredients	<1
CR	Water	75-100
	Carboxylic Acid	1-4
	Surfactant	1-4
	Organic Salt	1-4
	Hydrocarbon	1-4
	Solvent	1-4
	Minor Ingredients	<1
CS	Surfactant	50-74
	Hydrocarbon	10-24
	Citric Acid (Anhydrous)	5-9
	Carbohydrate	1-4
	Water	1-4
	Hydroxide	1-4
	Perfume	1-4
	Minor Ingredients	<1
CT	Water	75-100
	Carboxylic Acid	1-4
	Surfactant	1-4
	Organic Salt	1-4
	Hydrocarbon	1-4
	Solvent	1-4
	Minor Ingredients	<1

Components of AMCP Formulations Tested

Formulation Name	Formulation Components	Percentage
CU	Nta	25-49
	Surfactant	10-24
	Inorganic Salt	10-24
	Inorganic Salt	10-24
	Inorganic Salt	5-9
	Water	1-4
	Process Aid	1-4
	Minor Ingredients	<1
CV	Water	25-49
	Inorganic Salt	25-49
	Organic Salt	10-24
	Polyacrylate	5-9
	Surfactant	5-9
	Nadcc (Bleach)	1-4
	Minor Ingredients	<1
	Water	25-49
CW	Water	50-74
	Surfactant	24-49
	Solvent	5-9
	Salt	1-4
	Minor Ingredients	<1
CX	Surfactant	25-49
	Solvent	10-24
	Sodium Perborate Monohydrate (Bleach)	10-24
	Inorganic Salt	10-24
	Chelant	5-9
	Bleach Activator	5-9
	Water	1-4
	Ethoxylated Polymer	1-4
	Perfume	1-4
	Minor Ingredients	<1
CY	Water	25-49
	Surfactant	25-49
	Solvent	10-24
	Magnesium	1-4
	Minor Ingredients	<1
CZ	Water	75-100
	Solvent	1-4
	Hydrogen Peroxide	1-4
	Minor Ingredients	<1
DA	Water	75-100
	Hydrogen Peroxide	1-4
	Solvent	5-9
	Surfactant	1-4
	Minor Ingredients	<1
DB	Water	50-74
	Sodium Tripolyphosphate	10-24
	Inorganic Salt	5-9
	Hydroxide	1-4
	Inorganic Salt	1-4
	Sodium Hypochlorite	1-4
	Thickener	1-4
	Minor Ingredients	<1

Components of AMCP Formulations Tested

Formulation Name	Formulation Components	Percentage
DC	Water	50-74
	Surfactant	10-24
	Hydrogen Peroxide	5-9
	Organic Salt	1-4
	Carboxylic Acid	1-4
	Minor Ingredients	<1
DD	Water	50-74
	Surfactant	10-24
	Hydrogen Peroxide	5-9
	Organic Salt	1-4
	Solvent	1-4
	Carboxylic Acid	1-4
	Minor Ingredients	<1
DE	Water	75-100
	Hydrogen Peroxide	1-4
	Solvent	1-4
	Minor Ingredients	<1
DF	Siloxane and Silane	50-74
	Solvent	25-49
	Hydrocarbon	5-9
	Fragrance	1-4
	Ester	1-4
	Surfactant	1-4
	Minor Ingredients	<1
DG	Water	50-74
	Surfactant	25-49
	Salt	5-9
	Solvent	5-9
	Perfume	1-4
	Minor Ingredients	<1
DH	Water	50-74
	Surfactant	10-24
	Hydrogen Peroxide	5-9
	Organic Salt	1-4
	Carboxylic Acid	1-4
	Minor Ingredients	<1
DI	Water	25-49
	Surfactant	25-49
	Amide	10-24
	Inorganic Acid	1-4
	Minor Ingredients	<1
DJ	Siloxane and Silane	50-74
	Solvent	25-49
	Hydrocarbon	5-9
	Fragrance	5-9
	Ester	1-4
	Carboxylic Acid	1-4
	Minor Ingredients	<1

Components of AMCP Formulations Tested

Formulation Name	Formulation Components	Percentage
DK	Water	50-74
	Sodium Tripolyphosphate	10-24
	Inorganic Salt	5-9
	Hydroxide	1-4
	Hydroxide	1-4
	Sodium Hypochlorite	1-4
	Thickener	1-4
	Minor Ingredients	<1
EF, Cleaner/Degreaser	Water	65-70
	Glycol Ether	10-15
	Anionic Surfactant	1-5
	Inorganic Salt	1-5
	Chelator	1-5
	Nonionic Surfactant	5-10
	NaOH	1-5
	Dye	<1
EG, Bathroom Cleaner	Antimicrobial	<1
	Carrier	90-95
	Solvent	1-5
	Cleaning Agent	<1
	Solubilizer	<1

Annex II

In Vitro Data for Substances Tested in the BCOP Test Method

Annex II-1
BCOP Data Sorted by Reference.....C-161

Annex II-2
BCOP Data Sorted by Substance Name.....C-213

This page intentionally left blank

Annex II-1

BCOP Data Sorted by Reference

This page intentionally left blank

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
A		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	206.9	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AB		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	90	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AC		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	134.8	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AD		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	113.1	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AE		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	66.7	n.p.	n.p.	Cat I	Cat I	Cat II	AMCP BRD
AF		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	9.6	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD
AG		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	391.9	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AH		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	255.7	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AI		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	354.7	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AJ		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	357.1	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AK		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	444.3	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AL		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	353.6	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AM		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	135.8	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AN		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	113.5	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AO		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	216.2	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AP		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	393.3	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AQ		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	84.9	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AR		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	116.1	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AS		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	79.8	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AT		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	85.6	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AU		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	122.2	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AV		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	191.8	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AW		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	43.1	n.p.	n.p.	Cat II	Cat II	Cat II	AMCP BRD
AX		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	157.3	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AY		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	194.3	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
B		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	152.2	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
BB		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	2	n.p.	n.p.	Not Labeled	Not Labeled	Cat IV	AMCP BRD
BD		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	18.3	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
BE		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	15	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD
BF		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	63.5	n.p.	n.p.	Cat I	Cat I	Cat II	AMCP BRD
BJ		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	78.3	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
BJ		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	54.6	n.p.	n.p.	Cat II	Cat II	Cat II	AMCP BRD
BK		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	6.7	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD
BL		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	6	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD
BM		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	25.4	n.p.	n.p.	Cat II	Cat II	Cat II	AMCP BRD
BN		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	13.5	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD
BP		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	19.1	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD
BQ		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	33.6	n.p.	n.p.	Cat II	Cat II	Cat II	AMCP BRD
C		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	29.7	n.p.	n.p.	Cat II	Cat II	Cat II	AMCP BRD
CG		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	3.9	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD
CH		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	17.4	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD
D		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	187.7	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
E		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	196.2	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
EF		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	104.8	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
EG		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	71.8	n.p.	n.p.	Cat I	Cat I	Cat II	AMCP BRD
F		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	360.8	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
G		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	139.7	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
H		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	14	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD
I		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	0.6	n.p.	n.p.	Not Labeled	Not Labeled	Cat IV	AMCP BRD
J		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	7.7	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD
K		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	0.3	n.p.	n.p.	Not Labeled	Not Labeled	Cat IV	AMCP BRD
L		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	5.5	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD
M		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	55.7	n.p.	n.p.	Cat I	Cat I	Cat II	AMCP BRD
N		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	152.7	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
O		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	7.2	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD
P		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	1.1	n.p.	n.p.	Not Labeled	Not Labeled	Cat IV	AMCP BRD

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Q		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	13.5	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD
R		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	0.2	n.p.	n.p.	Not Labeled	Not Labeled	Cat IV	AMCP BRD
S		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	18.8	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD
T		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	1.8	n.p.	n.p.	Not Labeled	Not Labeled	Cat IV	AMCP BRD
U		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	3.4	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD
V		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	20.8	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD
W		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	5.7	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD
X		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	81.9	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
Y		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	74.9	n.p.	n.p.	Cat I	Cat I	Cat II	AMCP BRD
Z		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	31.6	n.p.	n.p.	Cat II	Cat II	Cat II	AMCP BRD
2-Chloro-2,4,4-trimethylpentane	-	liquid	100%	n.p.	-	4.0			0.004			4.1			Mild	Mild	Cat III	Bailey et al. (2004)
5-Ethylidene-2-norbornene	16219-75-3	liquid	100%	n.p.	-	5.7			0.207			8.8			Mild	Mild	Cat III	Bailey et al. (2004)
Alkyl phosphoric acid ester/ amine salt	-	liquid	100%	n.p.	-	37.7			3.577			91.3			Severe	Severe	Cat I	Bailey et al. (2004)
Aromatic hydrocarbon #1	-	liquid	100%	n.p.	-	2.7			0.000			2.7			Not Labeled	Not Labeled	Cat IV	Bailey et al. (2004)
Aromatic hydrocarbon #2	-	liquid	100%	n.p.	-	4.3			0.017			4.6			Mild	Mild	Cat III	Bailey et al. (2004)
Aryl phosponates	-	liquid	100%	n.p.	-	20.3			1.399			41.3			Moderate	Moderate	Cat II	Bailey et al. (2004)
Carboxylic acid amides	-	solid	100%	n.p.	-	10.7			1.125			27.5			Moderate	Moderate	Cat II	Bailey et al. (2004)
Clarified slurry oil	-	liquid	100%	n.p.	-	2.3			0.000			2.3			Not Labeled	Not Labeled	Cat IV	Bailey et al. (2004)
Cutting fluid (conc.) #1	-	liquid	100%	n.p.	-	3.3			0.001			3.5			Mild	Mild	Cat III	Bailey et al. (2004)
Cutting fluid (conc.) #2	-	liquid	100%	n.p.	-	4.3			0.038			4.9			Mild	Mild	Cat III	Bailey et al. (2004)
Ethylhexyl acid phosphate ester	-	liquid	100%	n.p.	-	117.3			0.880			130.5			Severe	Severe	Cat I	Bailey et al. (2004)
Methyl cyclopentadiene dimer	-	liquid	100%	n.p.	-	0.7			0.001			0.7			Not Labeled	Not Labeled	Cat IV	Bailey et al. (2004)
Petroleum wax	-	solid	100%	n.p.	-	0.3			-0.001			0.3			Not Labeled	Not Labeled	Cat IV	Bailey et al. (2004)
Polyalkenylsuccinate ester/ amine salt	-	liquid	100%	n.p.	-	2.3			0.000			2.3			Not Labeled	Not Labeled	Cat IV	Bailey et al. (2004)
Process oil	-	liquid	100%	n.p.	-	2.7			0.004			2.7			Not Labeled	Not Labeled	Cat IV	Bailey et al. (2004)
Thiadiazole alkyl derivative	-	liquid	100%	n.p.	-	7.3			0.237			10.9			Moderate	Moderate	Cat III	Bailey et al. (2004)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
1-Naphthalene acetic acid	86-87-3	solid	20%	96	1	119.4			0.095			120.8			Very severe	Very Severe	Cat II	Balls et al. (1995)
1-Naphthalene acetic acid	86-87-3	solid	20%	96	2	65.7			0.045			66.3			Severe			Balls et al. (1995)
1-Naphthalene acetic acid	86-87-3	solid	20%	96	3	41			0.065			42			Moderate			Balls et al. (1995)
1-Naphthalene acetic acid	86-87-3	solid	20%	96	4	86.67			0.137			88.73			Very severe			Balls et al. (1995)
1-Naphthalene acetic acid	86-87-3	solid	20%	96	5	70			0.168			72.5			Severe			Balls et al. (1995)
1-Naphthalene acetic acid, Na salt	61-31-4	solid	20%	95	1	73.3			4.177			136			Very severe	Very Severe	Cat I	Balls et al. (1995)
1-Naphthalene acetic acid, Na salt	61-31-4	solid	20%	95	2	83			4.124			144.9			Very severe			Balls et al. (1995)
1-Naphthalene acetic acid, Na salt	61-31-4	solid	20%	95	3	73			5.864			161			Very severe			Balls et al. (1995)
1-Naphthalene acetic acid, Na salt	61-31-4	solid	20%	95	4	108			3.55			161.2			Very severe			Balls et al. (1995)
1-Naphthalene acetic acid, Na salt	61-31-4	solid	20%	95	5	94.7			3.222			143			Very severe			Balls et al. (1995)
2,2-Dimethylbutanoic acid	595-37-9	liquid	100%	96	1	65			2.583			103.8			Very severe	Very Severe	Cat I	Balls et al. (1995)
2,2-Dimethylbutanoic acid	595-37-9	liquid	100%	96	2	58.3			3.78			115			Very severe			Balls et al. (1995)
2,2-Dimethylbutanoic acid	595-37-9	liquid	100%	96	3	62.7			4.601			131.7			Very severe			Balls et al. (1995)
2,2-Dimethylbutanoic acid	595-37-9	liquid	100%	96	4	84			3.803			130.26			Very severe			Balls et al. (1995)
2,2-Dimethylbutanoic acid	595-37-9	liquid	100%	96	5	37			2.783			78.8			Severe			Balls et al. (1995)
2,5-Dimethylhexanediol	110-03-2	solid	20%	99.5	1	12			0.415			18.2			Mild	Mild	Cat III	Balls et al. (1995)
2,5-Dimethylhexanediol	110-03-2	solid	20%	99.5	2	10.7			0.979			25.3			Moderate			Balls et al. (1995)
2,5-Dimethylhexanediol	110-03-2	solid	20%	99.5	3	6.7			0.925			20.5			Mild			Balls et al. (1995)
2,5-Dimethylhexanediol	110-03-2	solid	20%	99.5	4	21.33			0.68			31.533			Moderate			Balls et al. (1995)
2,5-Dimethylhexanediol	110-03-2	solid	20%	99.5	5	4.7			0.245			8.3			Mild			Balls et al. (1995)
2,6-Dichlorobenzoyl chloride	4659-45-4	liquid	100%	99	1	9			0.058			9.9			Mild	Mild	Cat III	Balls et al. (1995)
2,6-Dichlorobenzoyl chloride	4659-45-4	liquid	100%	99	2	10.3			0.059			11.2			Mild			Balls et al. (1995)
2,6-Dichlorobenzoyl chloride	4659-45-4	liquid	100%	99	3	9.7			0.078			10.8			Mild			Balls et al. (1995)
2,6-Dichlorobenzoyl chloride	4659-45-4	liquid	100%	99	4	14.33			0.007			14.43			Mild			Balls et al. (1995)
2,6-Dichlorobenzoyl chloride	4659-45-4	liquid	100%	99	5	5.4			0.012			5.6			Mild			Balls et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
2-Ethyl-1-hexanol	104-76-7	liquid	100%	99	2	9			1.279			28.2			Moderate	Moderate	Cat II	Balls et al. (1995)
2-Ethyl-1-hexanol	104-76-7	liquid	100%	99	3	4.3			1.761			30.7			Moderate			Balls et al. (1995)
2-Ethyl-1-hexanol	104-76-7	liquid	100%	99	4	7			3.347			58.71			Severe			Balls et al. (1995)
2-Ethyl-1-hexanol	104-76-7	liquid	100%	99	5	7			0.837			19.6			Mild			Balls et al. (1995)
4-Carboxybenzaldehyde	619-66-9	solid	20%	95	1	97.3			0.02			97.6			Very severe	Severe	Cat II	Balls et al. (1995)
4-Carboxybenzaldehyde	619-66-9	solid	20%	95	2	96.3			0.116			98.1			Very severe			Balls et al. (1995)
4-Carboxybenzaldehyde	619-66-9	solid	20%	95	3	57.3			0.012			57.5			Severe			Balls et al. (1995)
4-Carboxybenzaldehyde	619-66-9	solid	20%	95	4	64			0.022			64.33			Severe			Balls et al. (1995)
4-Carboxybenzaldehyde	619-66-9	solid	20%	95	5	72			0.128			73.9			Severe			Balls et al. (1995)
Acetone	67-64-1	liquid	100%	99	1	90.3			3.676			145.5			Very severe	Very Severe	Cat I	Balls et al. (1995)
Acetone	67-64-1	liquid	100%	99	2	83.7			2.389			119.5			Very severe			Balls et al. (1995)
Acetone	67-64-1	liquid	100%	99	3	55.7			4.315			120.4			Very severe			Balls et al. (1995)
Acetone	67-64-1	liquid	100%	99	4	94.33			2.492			131.72			Very severe			Balls et al. (1995)
Acetone	67-64-1	liquid	100%	99	5	69.3			1.942			98.4			Very severe			Balls et al. (1995)
Ammonium nitrate	6484-52-2	solid	20%	>99.9	1	6.3			0.132			8.3			Mild	Mild	Cat III	Balls et al. (1995)
Ammonium nitrate	6484-52-2	solid	20%	>99.9	2	6			0.026			6.4			Mild			Balls et al. (1995)
Ammonium nitrate	6484-52-2	solid	20%	>99.9	3	6			0.079			7.2			Mild			Balls et al. (1995)
Ammonium nitrate	6484-52-2	solid	20%	>99.9	4	11.34			0.698			21.82			Mild			Balls et al. (1995)
Ammonium nitrate	6484-52-2	solid	20%	>99.9	5	4.7			0.034			5.2			Mild			Balls et al. (1995)
Benzalkonium chloride (1 %)	8001-54-5	liquid	1%	98	1	59			3.588			112.8			Very severe	Very Severe	Cat I	Balls et al. (1995)
Benzalkonium chloride (1 %)	8001-54-5	liquid	1%	98	2	37			3.566			90.5			Very severe			Balls et al. (1995)
Benzalkonium chloride (1 %)	8001-54-5	liquid	1%	98	3	34.3			4.336			99.4			Very severe			Balls et al. (1995)
Benzalkonium chloride (1 %)	8001-54-5	liquid	1%	98	4	22			2.699			62.49			Severe			Balls et al. (1995)
Benzalkonium chloride (1 %)	8001-54-5	liquid	1%	98	5	38			2.706			78.6			Severe			Balls et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Benzalkonium chloride (10%)	8001-54-5	liquid	10%	98	1	75.3			4.456			142.2			Very severe	Very Severe	Cat I	Balls et al. (1995)
Benzalkonium chloride (10%)	8001-54-5	liquid	10%	98	2	79.3			5.223			157.7			Very severe			Balls et al. (1995)
Benzalkonium chloride (10%)	8001-54-5	liquid	10%	98	3	61.7			4.142			123.8			Very severe			Balls et al. (1995)
Benzalkonium chloride (10%)	8001-54-5	liquid	10%	98	4	63			4.967			137.5			Very severe			Balls et al. (1995)
Benzalkonium chloride (10%)	8001-54-5	liquid	10%	98	5	74.7			3.096			121.1			Very severe			Balls et al. (1995)
Benzalkonium chloride (5%)	8001-54-5	liquid	5%	98	1	126.6			3.264			126.6			Very severe	Very Severe	Cat I	Balls et al. (1995)
Benzalkonium chloride (5%)	8001-54-5	liquid	5%	98	2	163.7			6.599			163.7			Very severe			Balls et al. (1995)
Benzalkonium chloride (5%)	8001-54-5	liquid	5%	98	3	110.7			3.891			110.7			Very severe			Balls et al. (1995)
Benzalkonium chloride (5%)	8001-54-5	liquid	5%	98	4	130.41			4.338			130.41			Very severe			Balls et al. (1995)
Benzalkonium chloride (5%)	8001-54-5	liquid	5%	98	5	111.1			3.117			111.1			Very severe			Balls et al. (1995)
Benzoyl-L-tartaric acid	2743-38-6	solid	20%													Severe	Cat I	Balls et al. (1995)
Benzoyl-L-tartaric acid	2743-38-6	solid	20%															Balls et al. (1995)
Benzoyl-L-tartaric acid	2743-38-6	solid	20%															Balls et al. (1995)
Benzoyl-L-tartaric acid	2743-38-6	solid	20%															Balls et al. (1995)
Benzoyl-L-tartaric acid	2743-38-6	solid	20%															Balls et al. (1995)
Butyl acetate	123-86-4	liquid	100%	99	1	9			2.7			49.5			Moderate	Moderate	Cat II	Balls et al. (1995)
Butyl acetate	123-86-4	liquid	100%	99	2	7.7			1.989			37.5			Moderate			Balls et al. (1995)
Butyl acetate	123-86-4	liquid	100%	99	3	5.7			2.546			43.9			Moderate			Balls et al. (1995)
Butyl acetate	123-86-4	liquid	100%	99	4	5			1.257			23.86			Mild			Balls et al. (1995)
Butyl acetate	123-86-4	liquid	100%	99	5	2.3			1.051			18.1			Mild			Balls et al. (1995)
Captan 90 concentrate	133-06-2	solid	20%	90	1	28			-0.008			27.8			Moderate	Moderate	Cat II	Balls et al. (1995)
Captan 90 concentrate	133-06-2	solid	20%	90	2	26.3			0.055			27.2			Moderate			Balls et al. (1995)
Captan 90 concentrate	133-06-2	solid	20%	90	3	34.7			0.007			34.8			Moderate			Balls et al. (1995)
Captan 90 concentrate	133-06-2	solid	20%	90	4	102			0.061			102.918			Very severe			Balls et al. (1995)
Captan 90 concentrate	133-06-2	solid	20%	90	5	26.3			0.004			26.4			Moderate			Balls et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Cetylpyridinium bromide (0.1%)	140-72-7	liquid	0.10%	98	1	6.7			0.293			11			Mild	Mild	Cat III	Balls et al. (1995)
Cetylpyridinium bromide (0.1%)	140-72-7	liquid	0.10%	98	2	1.7			0.163			4.1			Mild			Balls et al. (1995)
Cetylpyridinium bromide (0.1%)	140-72-7	liquid	0.10%	98	3	3			0.606			12.1			Mild			Balls et al. (1995)
Cetylpyridinium bromide (0.1%)	140-72-7	liquid	0.10%	98	4	3.33			0.066			4.33			Mild			Balls et al. (1995)
Cetylpyridinium bromide (0.1%)	140-72-7	liquid	0.10%	98	5	6.3			0.543			14.5			Mild			Balls et al. (1995)
Cetylpyridinium bromide (10%)	140-72-7	liquid	10%	98	1	22.7			1.389			43.5			Moderate	Very Severe	Cat I	Balls et al. (1995)
Cetylpyridinium bromide (10%)	140-72-7	liquid	10%	98	2	27.7			4.128			89.6			Very severe			Balls et al. (1995)
Cetylpyridinium bromide (10%)	140-72-7	liquid	10%	98	3	24.7			3.759			81			Very severe			Balls et al. (1995)
Cetylpyridinium bromide (10%)	140-72-7	liquid	10%	98	4	17			3.97			71.22			Severe			Balls et al. (1995)
Cetylpyridinium bromide (10%)	140-72-7	liquid	10%	98	5	23			3.58			76.7			Severe			Balls et al. (1995)
Cetylpyridinium bromide (6%)	140-72-7	liquid	6%	98	1	31.7			2.705			72.2			Severe	Severe	Cat II	Balls et al. (1995)
Cetylpyridinium bromide (6%)	140-72-7	liquid	6%	98	2	38.3			3.195			86.3			Very severe			Balls et al. (1995)
Cetylpyridinium bromide (6%)	140-72-7	liquid	6%	98	3	18.3			3.015			63.6			Severe			Balls et al. (1995)
Cetylpyridinium bromide (6%)	140-72-7	liquid	6%	98	4	25.33			2.892			68.72			Severe			Balls et al. (1995)
Cetylpyridinium bromide (6%)	140-72-7	liquid	6%	98	5	34			2.097			65.4			Severe			Balls et al. (1995)
Chlorhexidine	55-56-1	solid	20%	n.p.[12]	1	141			0.399			147			Very severe	Very Severe	Cat I	Balls et al. (1995)
Chlorhexidine	55-56-1	solid	20%	n.p.	2	124			-0.071			122.9			Very severe			Balls et al. (1995)
Chlorhexidine	55-56-1	solid	20%	n.p.	3	96.3			0.062			97.3			Very severe			Balls et al. (1995)
Chlorhexidine	55-56-1	solid	20%	n.p.	4	97.66			0.277			101.78			Very severe			Balls et al. (1995)
Chlorhexidine	55-56-1	solid	20%	n.p.	5	98.7			0.189			101.5			Very severe			Balls et al. (1995)
Cyclohexanol	108-93-0	liquid	100%	97	1	18.3			4.442			85			Very severe	Moderate	Cat II	Balls et al. (1995)
Cyclohexanol	108-93-0	liquid	100%	97	2	7.3			2.838			49.9			Moderate			Balls et al. (1995)
Cyclohexanol	108-93-0	liquid	100%	97	3	12			3.87			70.1			Severe			Balls et al. (1995)
Cyclohexanol	108-93-0	liquid	100%	97	4	11.66			2.71			52.24			Moderate			Balls et al. (1995)
Cyclohexanol	108-93-0	liquid	100%	97	5	7			2.392			43.2			Moderate			Balls et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Dibenzyl phosphate	1623-08-1	solid	20%	99	1	304.3			-0.017			304.1			Very severe	Very Severe	Cat I	Balls et al. (1995)
Dibenzyl phosphate	1623-08-1	solid	20%	99	2	389.3			0.117			391.1			Very severe			Balls et al. (1995)
Dibenzyl phosphate	1623-08-1	solid	20%	99	3	418			-0.002			418			Very severe			Balls et al. (1995)
Dibenzyl phosphate	1623-08-1	solid	20%	99	4	467			-0.016			467.09			Very severe			Balls et al. (1995)
Dibenzyl phosphate	1623-08-1	solid	20%	99	5	304			0.234			307.5			Very severe			Balls et al. (1995)
Ethanol	64-17-5	liquid	100%	n.p.	1	31			2.893			74.4			Severe	Severe	Cat I	Balls et al. (1995)
Ethanol	64-17-5	liquid	100%	n.p.	2	21.3			2.123			53.2			Moderate			Balls et al. (1995)
Ethanol	64-17-5	liquid	100%	n.p.	3	16.3			3.134			63.3			Severe			Balls et al. (1995)
Ethanol	64-17-5	liquid	100%	n.p.	4	36			4.134			98.01			Very severe			Balls et al. (1995)
Ethanol	64-17-5	liquid	100%	n.p.	5	30			2.277			64.2			Severe			Balls et al. (1995)
Ethyl acetate	141-78-6	liquid	100%	99	1	8.7			0.737			19.7			Mild	Moderate	Cat II	Balls et al. (1995)
Ethyl acetate	141-78-6	liquid	100%	99	2	5.7			1.513			28.4			Moderate			Balls et al. (1995)
Ethyl acetate	141-78-6	liquid	100%	99	3	9			2.543			47.1			Moderate			Balls et al. (1995)
Ethyl acetate	141-78-6	liquid	100%	99	4	13.33			2.065			44.31			Moderate			Balls et al. (1995)
Ethyl acetate	141-78-6	liquid	100%	99	5	11			0.64			20.6			Mild			Balls et al. (1995)
Ethyl trimethyl acetate	3938-95-2	liquid	100%	99	1	10.3			1.136			27.4			Moderate	Mild	Cat III	Balls et al. (1995)
Ethyl trimethyl acetate	3938-95-2	liquid	100%	99	2	5			1.916			33.7			Moderate			Balls et al. (1995)
Ethyl trimethyl acetate	3938-95-2	liquid	100%	99	3	1.3			0.609			10.5			Mild			Balls et al. (1995)
Ethyl trimethyl acetate	3938-95-2	liquid	100%	99	4	5.33			0.22			8.633			Mild			Balls et al. (1995)
Ethyl trimethyl acetate	3938-95-2	liquid	100%	99	5	3.6			0.357			9			Mild			Balls et al. (1995)
Ethyl-2-methylacetoacetate	609-14-3	liquid	100%	97	1	26.7			0.052			27.5			Moderate	Mild	Cat III	Balls et al. (1995)
Ethyl-2-methylacetoacetate	609-14-3	liquid	100%	97	2	14.3			-0.014			14.1			Mild			Balls et al. (1995)
Ethyl-2-methylacetoacetate	609-14-3	liquid	100%	97	3	5.7			-0.012			5.5			Mild			Balls et al. (1995)
Ethyl-2-methylacetoacetate	609-14-3	liquid	100%	97	4	5.33			0.014			5.543			Mild			Balls et al. (1995)
Ethyl-2-methylacetoacetate	609-14-3	liquid	100%	97	5	18.7			0.061			19.6			Mild			Balls et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Fomesafen	72128-02-0	solid	20%	97.5	2	4.3			9.837			151.9			Very severe	Severe	Cat II	Balls et al. (1995)
Fomesafen	72128-02-0	solid	20%	97.5	3	6.3			3.904			64.9			Severe			Balls et al. (1995)
Fomesafen	72128-02-0	solid	20%	97.5	4	13			0.668			23.023			Mild			Balls et al. (1995)
Fomesafen	72128-02-0	solid	20%	97.5	5	5.7			0.834			18.2			Mild			Balls et al. (1995)
gamma-Butyrolactone	96-48-0	liquid	100%	>99	1	37.3			3.553			90.6			Very severe	Severe	Cat II	Balls et al. (1995)
gamma-Butyrolactone	96-48-0	liquid	100%	>99	2	22.7			0.682			32.9			Moderate			Balls et al. (1995)
gamma-Butyrolactone	96-48-0	liquid	100%	>99	3	22			0.63			31.5			Moderate			Balls et al. (1995)
gamma-Butyrolactone	96-48-0	liquid	100%	>99	4	48.67			2.192			81.55			Very severe			Balls et al. (1995)
gamma-Butyrolactone	96-48-0	liquid	100%	>99	5	31.7			2.357			67.1			Severe			Balls et al. (1995)
Glycerol	56-81-5	liquid	100%	>99.5	1	-2			-0.001			-2			Not Labeled	Not Labeled	Cat IV	Balls et al. (1995)
Glycerol	56-81-5	liquid	100%	>99.5	2	-0.7			0.029			-0.2			Not Labeled			Balls et al. (1995)
Glycerol	56-81-5	liquid	100%	>99.5	3	0			0.018			0.3			Not Labeled			Balls et al. (1995)
Glycerol	56-81-5	liquid	100%	>99.5	4	3			0.005			3.08			Mild			Balls et al. (1995)
Glycerol	56-81-5	liquid	100%	>99.5	5	0			0.01			0.1			Not Labeled			Balls et al. (1995)
Imidazole	288-32-4	solid	20%	99	1	68.3			3.232			116.8			Very severe	Severe	Cat I	Balls et al. (1995)
Imidazole	288-32-4	solid	20%	99	2	93			2.724			133.9			Very severe			Balls et al. (1995)
Imidazole	288-32-4	solid	20%	99	3	62.3			2.741			103.4			Very severe			Balls et al. (1995)
Imidazole	288-32-4	solid	20%	99	4	97.34			1.424			118.7			Very severe			Balls et al. (1995)
Imidazole	288-32-4	solid	20%	99	5	54.3			2.431			90.8			Very severe			Balls et al. (1995)
Isobutanol	78-83-1	liquid	100%	99.9	1	17			2.494			54.4			Moderate	Moderate	Cat II	Balls et al. (1995)
Isobutanol	78-83-1	liquid	100%	99.9	2	20			3.598			74			Severe			Balls et al. (1995)
Isobutanol	78-83-1	liquid	100%	99.9	3	19			3.248			67.7			Severe			Balls et al. (1995)
Isobutanol	78-83-1	liquid	100%	99.9	4	26			1.052			41.78			Moderate			Balls et al. (1995)
Isobutanol	78-83-1	liquid	100%	99.9	5	21.4			1.39			42.2			Moderate			Balls et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Isopropanol	67-63-0	liquid	100%	99.9	1	11.7			1.868			39.7			Moderate	Severe	Cat II	Balls et al. (1995)
Isopropanol	67-63-0	liquid	100%	99.9	2	23.3			2.409			59.5			Severe			Balls et al. (1995)
Isopropanol	67-63-0	liquid	100%	99.9	3	16			3.755			72.3			Severe			Balls et al. (1995)
Isopropanol	67-63-0	liquid	100%	99.9	4	30.66			3.189			78.5			Severe			Balls et al. (1995)
Isopropanol	67-63-0	liquid	100%	99.9	5	18.3			1.4			39.3			Moderate			Balls et al. (1995)
L-Aspartic acid	70-47-3	solid	20%	100	1	2			-0.011			1.8			Not Labeled	Not Labeled	Cat IV	Balls et al. (1995)
L-Aspartic acid	70-47-3	solid	20%	100	2	1.7			-0.107			0.1			Not Labeled			Balls et al. (1995)
L-Aspartic acid	70-47-3	solid	20%	100	3	2.7			-0.003			2.6			Not Labeled			Balls et al. (1995)
L-Aspartic acid	70-47-3	solid	20%	100	4	0.33			0.03			0.788			Not Labeled			Balls et al. (1995)
L-Aspartic acid	70-47-3	solid	20%	100	5	0			0.082			1.2			Not Labeled			Balls et al. (1995)
Maneb	12427-38-2	solid	20%	90	2	17			-0.008			16.9			Mild	Mild	Cat II	Balls et al. (1995)
Maneb	12427-38-2	solid	20%	90	3	21			-0.002			21			Mild			Balls et al. (1995)
Maneb	12427-38-2	solid	20%	90	4	56.33			0.495			63.76			Severe			Balls et al. (1995)
Maneb	12427-38-2	solid	20%	90	5	33.3			0.029			33.8			Moderate			Balls et al. (1995)
Methyl acetate	79-20-9	liquid	100%	98	1	51.6			1.301			71.2			Severe	Moderate	Cat II	Balls et al. (1995)
Methyl acetate	79-20-9	liquid	100%	98	2	42			0.299			46.5			Moderate			Balls et al. (1995)
Methyl acetate	79-20-9	liquid	100%	98	3	38.3			0.887			51.6			Moderate			Balls et al. (1995)
Methyl acetate	79-20-9	liquid	100%	98	4	43.1			0.72			53.9			Moderate			Balls et al. (1995)
Methyl acetate	79-20-9	liquid	100%	98	5	45.3			0.384			51.1			Moderate			Balls et al. (1995)
Methyl cyanoacetate	105-34-0	liquid	100%	99	1	16.3			0.002			16.3			Mild	Mild	Cat III	Balls et al. (1995)
Methyl cyanoacetate	105-34-0	liquid	100%	99	2	6.7			-0.052			5.9			Mild			Balls et al. (1995)
Methyl cyanoacetate	105-34-0	liquid	100%	99	3	10.3			-0.015			10.1			Mild			Balls et al. (1995)
Methyl cyanoacetate	105-34-0	liquid	100%	99	4	17.33			0.013			17.53			Mild			Balls et al. (1995)
Methyl cyanoacetate	105-34-0	liquid	100%	99	5	11			-0.003			11			Mild			Balls et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Methyl ethyl ketone	78-93-3	liquid	100%	99	1	68			1.665			93			Very severe	Severe	Cat II	Balls et al. (1995)
Methyl ethyl ketone	78-93-3	liquid	100%	99	2	51.3			1.069			67.4			Severe			Balls et al. (1995)
Methyl ethyl ketone	78-93-3	liquid	100%	99	3	34			1.212			52.2			Moderate			Balls et al. (1995)
Methyl ethyl ketone	78-93-3	liquid	100%	99	4	58			1.38			78.71			Severe			Balls et al. (1995)
Methyl ethyl ketone	78-93-3	liquid	100%	99	5	51.7			0.607			60.8			Severe			Balls et al. (1995)
Methyl isobutyl ketone	108-10-1	liquid	100%	98	1	4.7			0.273			8.8			Mild	Mild	Cat III	Balls et al. (1995)
Methyl isobutyl ketone	108-10-1	liquid	100%	98	2	8.7			0.759			20.1			Mild			Balls et al. (1995)
Methyl isobutyl ketone	108-10-1	liquid	100%	98	3	5.7			0.307			10.3			Mild			Balls et al. (1995)
Methyl isobutyl ketone	108-10-1	liquid	100%	98	4	8			0.35			13.25			Mild			Balls et al. (1995)
Methyl isobutyl ketone	108-10-1	liquid	100%	98	5	5.7			0.305			10.3			Mild			Balls et al. (1995)
Methylcyclopentane	96-37-7	liquid	100%	>99	1	1.3			0.169			3.8			Mild	Not Labeled	Cat IV	Balls et al. (1995)
Methylcyclopentane	96-37-7	liquid	100%	>99	2	2.3			0.152			4.6			Mild			Balls et al. (1995)
Methylcyclopentane	96-37-7	liquid	100%	>99	3	0.3			0.071			1.4			Not Labeled			Balls et al. (1995)
Methylcyclopentane	96-37-7	liquid	100%	>99	4	1			0.047			1.71			Not Labeled			Balls et al. (1995)
Methylcyclopentane	96-37-7	liquid	100%	>99	5	0.3			0.161			2.7			Not Labeled			Balls et al. (1995)
n-Hexanol	111-27-3	liquid	100%	98	1	17.7			3.591			71.5			Severe	Severe/Very Severe	Cat II	Balls et al. (1995)
n-Hexanol	111-27-3	liquid	100%	98	2	16			4.509			83.6			Very severe			Balls et al. (1995)
n-Hexanol	111-27-3	liquid	100%	98	3	7			3.746			63.2			Severe			Balls et al. (1995)
n-Hexanol	111-27-3	liquid	100%	98	4	15.33			2.191			48.19			Moderate			Balls et al. (1995)
n-Hexanol	111-27-3	liquid	100%	98	5	10.7			2.145			42.9			Moderate			Balls et al. (1995)
n-Octanol	111-87-5	liquid	100%	>99	1	11			2.159			43.4			Moderate	Moderate	Cat II	Balls et al. (1995)
n-Octanol	111-87-5	liquid	100%	>99	2	13			4.392			78.9			Severe			Balls et al. (1995)
n-Octanol	111-87-5	liquid	100%	>99	3	10			1.984			39.8			Moderate			Balls et al. (1995)
n-Octanol	111-87-5	liquid	100%	>99	4	6			0.569			14.54			Mild			Balls et al. (1995)
n-Octanol	111-87-5	liquid	100%	>99	5	6			1.464			28			Moderate			Balls et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Parafluoraniline	371-40-4	liquid	100%	99	1	17.3			0.809			29.5			Moderate	Moderate	Cat II	Balls et al. (1995)
Parafluoraniline	371-40-4	liquid	100%	99	2	11.3			1.006			26.4			Moderate			Balls et al. (1995)
Parafluoraniline	371-40-4	liquid	100%	99	3	18.7			1.474			40.8			Moderate			Balls et al. (1995)
Parafluoraniline	371-40-4	liquid	100%	99	4	18			0.8996			31.82			Moderate			Balls et al. (1995)
Parafluoraniline	371-40-4	liquid	100%	99	5	13.3			0.679			23.5			Moderate			Balls et al. (1995)
Polyethylene glycol 400	25322-68-3	liquid	100%	n.p.	1	0.3			0.019			0.6			Not Labeled	Not Labeled	Cat IV	Balls et al. (1995)
Polyethylene glycol 400	25322-68-3	liquid	100%	n.p.	2	2			0.036			2.5			Not Labeled			Balls et al. (1995)
Polyethylene glycol 400	25322-68-3	liquid	100%	n.p.	3	-1.7			0.021			-1.3			Not Labeled			Balls et al. (1995)
Polyethylene glycol 400	25322-68-3	liquid	100%	n.p.	4	1			0.005			1.08			Not Labeled			Balls et al. (1995)
Polyethylene glycol 400	25322-68-3	liquid	100%	n.p.	5	2.7			0.01			2.8			Not Labeled			Balls et al. (1995)
Potassium cyanate	590-28-3	solid	20%	97	1	8.7			0.499			16.2			Mild	Mild	Cat III	Balls et al. (1995)
Potassium cyanate	590-28-3	solid	20%	97	2	11			0.793			22.9			Mild			Balls et al. (1995)
Potassium cyanate	590-28-3	solid	20%	97	3	8.3			0.248			12			Mild			Balls et al. (1995)
Potassium cyanate	590-28-3	solid	20%	97	4	7			0.692			17.38			Mild			Balls et al. (1995)
Potassium cyanate	590-28-3	solid	20%	97	5	3			0.234			6.5			Mild			Balls et al. (1995)
Promethazine hydrochloride	58-33-3	solid	20%	98	1	120.7			-0.022			120.3			Very severe	Severe	Cat I	Balls et al. (1995)
Promethazine hydrochloride	58-33-3	solid	20%	98	2	87.7			-0.234			84.2			Very severe			Balls et al. (1995)
Promethazine hydrochloride	58-33-3	solid	20%	98	3	125			0.044			125.7			Very severe			Balls et al. (1995)
Promethazine hydrochloride	58-33-3	solid	20%	98	4	121.33			0.051			123.09			Very severe			Balls et al. (1995)
Promethazine hydrochloride	58-33-3	solid	20%	98	5	153.7			0.011			153.8			Very severe			Balls et al. (1995)
Pyridine	110-86-1	liquid	100%	>99.9	1	73.7			4.468			140.7			Very severe	Severe	Cat I	Balls et al. (1995)
Pyridine	110-86-1	liquid	100%	>99.9	2	83.7			4.117			145.4			Very severe			Balls et al. (1995)
Pyridine	110-86-1	liquid	100%	>99.9	3	61			4.763			132.4			Very severe			Balls et al. (1995)
Pyridine	110-86-1	liquid	100%	>99.9	4	87.33			7.445			199.02			Very severe			Balls et al. (1995)
Pyridine	110-86-1	liquid	100%	>99.9	5	74.7			3.204			122.7			Very severe			Balls et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Quinacrine	69-05-6	solid	20%	n.p.	1	1			-0.047			0.3			Not Labeled	Mild	Cat III	Balls et al. (1995)
Quinacrine	69-05-6	solid	20%	n.p.	2	0.3			0.002			0.4			Not Labeled			Balls et al. (1995)
Quinacrine	69-05-6	solid	20%	n.p.	3	1.7			0.028			2.1			Not Labeled			Balls et al. (1995)
Quinacrine	69-05-6	solid	20%	n.p.	4	2.34			-0.033			1.85			Not Labeled			Balls et al. (1995)
Quinacrine	69-05-6	solid	20%	n.p.	5	2			0.07			3.1			Mild			Balls et al. (1995)
Sodium hydroxide (1%)	1310-73-2	liquid	1%	reagent grade	1	100.3			4.471			167.4			Very severe	Very Severe	Cat I	Balls et al. (1995)
Sodium hydroxide (1%)	1310-73-2	liquid	1%	reagent grade	2	80.7			3.504			133.2			Very severe			Balls et al. (1995)
Sodium hydroxide (1%)	1310-73-2	liquid	1%	reagent grade	3	88.7			3.856			146.5			Very severe			Balls et al. (1995)
Sodium hydroxide (1%)	1310-73-2	liquid	1%	reagent grade	4	116.66			3.628			171.08			Very severe			Balls et al. (1995)
Sodium hydroxide (1%)	1310-73-2	liquid	1%	reagent grade	5	88			2.888			132.3			Very severe			Balls et al. (1995)
Sodium hydroxide (10%)	1310-73-2	liquid	10%	reagent grade	1	232.3			3.53			285.2			Very severe	Severe	Cat I	Balls et al. (1995)
Sodium hydroxide (10%)	1310-73-2	liquid	10%	reagent grade	2	173.3			3.382			224.1			Very severe			Balls et al. (1995)
Sodium hydroxide (10%)	1310-73-2	liquid	10%	reagent grade	3	197			3.849			254.7			Very severe			Balls et al. (1995)
Sodium hydroxide (10%)	1310-73-2	liquid	10%	reagent grade	4	283			4.329			348.27			Very severe			Balls et al. (1995)
Sodium hydroxide (10%)	1310-73-2	liquid	10%	reagent grade	5	197.3			3.321			247.2			Very severe			Balls et al. (1995)
Sodium lauryl sulfate (15 %)	151-21-3	liquid	15%	98	1	4			2.884			47.3			Moderate	Mild	Cat II	Balls et al. (1995)
Sodium lauryl sulfate (15 %)	151-21-3	liquid	15%	98	2	6			5.801			93			Very severe			Balls et al. (1995)
Sodium lauryl sulfate (15 %)	151-21-3	liquid	15%	98	3	3.3			3.988			63.2			Severe			Balls et al. (1995)
Sodium lauryl sulfate (15 %)	151-21-3	liquid	15%	98	4	1.66			3.862			59.61			Severe			Balls et al. (1995)
Sodium lauryl sulfate (15 %)	151-21-3	liquid	15%	98	5	7.7			3.042			53.3			Moderate			Balls et al. (1995)
Sodium lauryl sulfate (3 %)	151-21-3	liquid	3%	98	1	12.3			1.29			31.7			Moderate	Moderate	Cat II	Balls et al. (1995)
Sodium lauryl sulfate (3 %)	151-21-3	liquid	3%	98	2	3.3			1.892			31.7			Moderate			Balls et al. (1995)
Sodium lauryl sulfate (3 %)	151-21-3	liquid	3%	98	3	0.3			1.801			27.3			Moderate			Balls et al. (1995)
Sodium lauryl sulfate (3 %)	151-21-3	liquid	3%	98	4	6			1.348			26.22			Moderate			Balls et al. (1995)
Sodium lauryl sulfate (3 %)	151-21-3	liquid	3%	98	5	0			0.82			12.3			Mild			Balls et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Sodium oxalate	62-76-0	solid	20%	>99	1	1.3			0.054			2.1			Not Labeled	Mild	Cat III	Balls et al. (1995)
Sodium oxalate	62-76-0	solid	20%	>99	2	6.7			0.059			7.6			Mild			Balls et al. (1995)
Sodium oxalate	62-76-0	solid	20%	>99	3	3			0.187			5.8			Mild			Balls et al. (1995)
Sodium oxalate	62-76-0	solid	20%	>99	4	43			0.556			49.59			Moderate			Balls et al. (1995)
Sodium oxalate	62-76-0	solid	20%	>99	5	4			0.081			4.9			Mild			Balls et al. (1995)
Sodium perborate	10486-00-7	solid	20%	98.6	1	10			8.908			143.6			Very severe	Very Severe	Cat I	Balls et al. (1995)
Sodium perborate	10486-00-7	solid	20%	98.6	2	13.7			6.982			118.4			Very severe			Balls et al. (1995)
Sodium perborate	10486-00-7	solid	20%	98.6	3	10			5.749			96.2			Very severe			Balls et al. (1995)
Sodium perborate	10486-00-7	solid	20%	98.6	4	11			3.568			64.531			Severe			Balls et al. (1995)
Sodium perborate	10486-00-7	solid	20%	98.6	5	9.7			3.547			62.9			Severe			Balls et al. (1995)
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	97	1	24			-0.023			23.6			Mild	Mild	Cat III	Balls et al. (1995)
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	97	2	8.3			-0.027			7.9			Mild			Balls et al. (1995)
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	97	3	14.3			-0.008			14.2			Mild			Balls et al. (1995)
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	97	4	21.33			-0.045			20.65			Mild			Balls et al. (1995)
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	97	5	6			0.19			8.9			Mild			Balls et al. (1995)
Thiourea	62-56-6	solid	20%	>99	1	88			4.095			149.4			Very severe	Severe	Cat I	Balls et al. (1995)
Thiourea	62-56-6	solid	20%	>99	2	106.3			2.19			139.2			Very severe			Balls et al. (1995)
Thiourea	62-56-6	solid	20%	>99	3	82			3.572			135.6			Very severe			Balls et al. (1995)
Thiourea	62-56-6	solid	20%	>99	4	81.01			3.76			137.44			Very severe			Balls et al. (1995)
Thiourea	62-56-6	solid	20%	>99	5	74			1.671			99.1			Very severe			Balls et al. (1995)
Toluene	108-88-3	liquid	100%	99	1	9.3			2.26			43.3			Moderate	Moderate	Cat II	Balls et al. (1995)
Toluene	108-88-3	liquid	100%	99	2	6			1.813			33.2			Moderate			Balls et al. (1995)
Toluene	108-88-3	liquid	100%	99	3	5.3			2.122			37.2			Moderate			Balls et al. (1995)
Toluene	108-88-3	liquid	100%	99	4	2			2.427			38.41			Moderate			Balls et al. (1995)
Toluene	108-88-3	liquid	100%	99	5	4			1.473			26.1			Moderate			Balls et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Trichloroacetic acid (3%)	76-03-9	liquid	3%	reagent grade	1	79.3			0.173			81.9			Very severe	Severe/Very Severe	Cat I	Balls et al. (1995)
Trichloroacetic acid (3%)	76-03-9	liquid	3%	reagent grade	2	49			0.053			49.8			Moderate			Balls et al. (1995)
Trichloroacetic acid (3%)	76-03-9	liquid	3%	reagent grade	3	73.7			0.111			75.3			Severe			Balls et al. (1995)
Trichloroacetic acid (3%)	76-03-9	liquid	3%	reagent grade	4	92.33			0.042			92.97			Very severe			Balls et al. (1995)
Trichloroacetic acid (3%)	76-03-9	liquid	3%	reagent grade	5	78.4			0.067			79.3			Severe			Balls et al. (1995)
Trichloroacetic acid (30%)	76-03-9	liquid	30%	reagent grade	1	228			2.93			272			Very severe	Very Severe	Cat I	Balls et al. (1995)
Trichloroacetic acid (30%)	76-03-9	liquid	30%	reagent grade	2	154.7			4.687			225			Very severe			Balls et al. (1995)
Trichloroacetic acid (30%)	76-03-9	liquid	30%	reagent grade	3	245.3			3.44			296.9			Very severe			Balls et al. (1995)
Trichloroacetic acid (30%)	76-03-9	liquid	30%	reagent grade	4	277			3.072			323.08			Very severe			Balls et al. (1995)
Trichloroacetic acid (30%)	76-03-9	liquid	30%	reagent grade	5	157			3.115			203.7			Very severe			Balls et al. (1995)
Triton X-100 (10%)	9002-93-1	liquid	10%	98	1	6			5.312			85.7			Very severe	Severe/Very Severe	Cat I	Balls et al. (1995)
Triton X-100 (10%)	9002-93-1	liquid	10%	98	2	6.7			4.624			76			Severe			Balls et al. (1995)
Triton X-100 (10%)	9002-93-1	liquid	10%	98	3	6			5.337			86.1			Very severe			Balls et al. (1995)
Triton X-100 (10%)	9002-93-1	liquid	10%	98	4	3.33			3.617			57.58			Severe			Balls et al. (1995)
Triton X-100 (10%)	9002-93-1	liquid	10%	98	5	7.7			2.567			46.2			Moderate			Balls et al. (1995)
Triton X-100 (5 %)	9002-93-1	liquid	5%	98	1	5.3			4.6			74.3			Severe	Severe	Cat I	Balls et al. (1995)
Triton X-100 (5 %)	9002-93-1	liquid	5%	98	2	8.3			6.553			106.6			Very severe			Balls et al. (1995)
Triton X-100 (5 %)	9002-93-1	liquid	5%	98	3	3.7			5.099			80.2			Very severe			Balls et al. (1995)
Triton X-100 (5 %)	9002-93-1	liquid	5%	98	4	5			4.79			76.79			Very severe			Balls et al. (1995)
Triton X-100 (5 %)	9002-93-1	liquid	5%	98	5	7.7			3.06			53.6			Moderate			Balls et al. (1995)
Tween 20	9005-64-5	liquid	n.p.	98	1	-0.7			0.006			-0.6			Not Labeled	Not Labeled	Cat IV	Balls et al. (1995)
Tween 20	9005-64-5	liquid	n.p.	98	2	-0.3			-0.052			-1.1			Not Labeled			Balls et al. (1995)
Tween 20	9005-64-5	liquid	n.p.	98	3	-2			0.026			-1.6			Not Labeled			Balls et al. (1995)
Tween 20	9005-64-5	liquid	n.p.	98	4	2.67			0.0003			2.711			Not Labeled			Balls et al. (1995)
Tween 20	9005-64-5	liquid	n.p.	98	5	0.1			0.026			0.4			Not Labeled			Balls et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
1,2,3-Trichloropropane	96-18-4	liquid	100%	n.p.	1							47			Moderate	Moderate	Cat II	Gautheron et al. (1994)
1,2,3-Trichloropropane	96-18-4	liquid	100%	n.p.	2							42			Moderate			Gautheron et al. (1994)
1,2,3-Trichloropropane	96-18-4	liquid	100%	n.p.	3							78			Severe			Gautheron et al. (1994)
1,2,3-Trichloropropane	96-18-4	liquid	100%	n.p.	4							28			Moderate			Gautheron et al. (1994)
1,2,3-Trichloropropane	96-18-4	liquid	100%	n.p.	5							42			Moderate			Gautheron et al. (1994)
1,2,3-Trichloropropane	96-18-4	liquid	100%	n.p.	6							47			Moderate			Gautheron et al. (1994)
1,2,3-Trichloropropane	96-18-4	liquid	100%	n.p.	7							48			Moderate			Gautheron et al. (1994)
1,2,3-Trichloropropane	96-18-4	liquid	100%	n.p.	8							24			Mild			Gautheron et al. (1994)
1,2,3-Trichloropropane	96-18-4	liquid	100%	n.p.	9							91			Severe			Gautheron et al. (1994)
1,2,3-Trichloropropane	96-18-4	liquid	100%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
1,2,3-Trichloropropane	96-18-4	liquid	100%	n.p.	11							28			Moderate			Gautheron et al. (1994)
1,2,3-Trichloropropane	96-18-4	liquid	100%	n.p.	12							47			Moderate			Gautheron et al. (1994)
1,2,4-Trimethylbenzene	95-63-6	liquid	100%	n.p.	1							25			Mild	Mild	Cat III	Gautheron et al. (1994)
1,2,4-Trimethylbenzene	95-63-6	liquid	100%	n.p.	2							14			Mild			Gautheron et al. (1994)
1,2,4-Trimethylbenzene	95-63-6	liquid	100%	n.p.	3							26			Moderate			Gautheron et al. (1994)
1,2,4-Trimethylbenzene	95-63-6	liquid	100%	n.p.	4							11			Mild			Gautheron et al. (1994)
1,2,4-Trimethylbenzene	95-63-6	liquid	100%	n.p.	5							27			Moderate			Gautheron et al. (1994)
1,2,4-Trimethylbenzene	95-63-6	liquid	100%	n.p.	6							7			Mild			Gautheron et al. (1994)
1,2,4-Trimethylbenzene	95-63-6	liquid	100%	n.p.	7							9			Mild			Gautheron et al. (1994)
1,2,4-Trimethylbenzene	95-63-6	liquid	100%	n.p.	8							15			Mild			Gautheron et al. (1994)
1,2,4-Trimethylbenzene	95-63-6	liquid	100%	n.p.	9							21			Mild			Gautheron et al. (1994)
1,2,4-Trimethylbenzene	95-63-6	liquid	100%	n.p.	10							10			Mild			Gautheron et al. (1994)
1,2,4-Trimethylbenzene	95-63-6	liquid	100%	n.p.	11							7			Mild			Gautheron et al. (1994)
1,2,4-Trimethylbenzene	95-63-6	liquid	100%	n.p.	12							21			Mild			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
1-Nitropropane	108-03-2	liquid	100%	n.p.	1							11			Mild	Mild	Cat III	Gautheron et al. (1994)
1-Nitropropane	108-03-2	liquid	100%	n.p.	2							8			Mild			Gautheron et al. (1994)
1-Nitropropane	108-03-2	liquid	100%	n.p.	3							9			Mild			Gautheron et al. (1994)
1-Nitropropane	108-03-2	liquid	100%	n.p.	4							4			Mild			Gautheron et al. (1994)
1-Nitropropane	108-03-2	liquid	100%	n.p.	5							6			Mild			Gautheron et al. (1994)
1-Nitropropane	108-03-2	liquid	100%	n.p.	6							7			Mild			Gautheron et al. (1994)
1-Nitropropane	108-03-2	liquid	100%	n.p.	7							6			Mild			Gautheron et al. (1994)
1-Nitropropane	108-03-2	liquid	100%	n.p.	8							6			Mild			Gautheron et al. (1994)
1-Nitropropane	108-03-2	liquid	100%	n.p.	9							17			Mild			Gautheron et al. (1994)
1-Nitropropane	108-03-2	liquid	100%	n.p.	10							4			Mild			Gautheron et al. (1994)
1-Nitropropane	108-03-2	liquid	100%	n.p.	11							6			Mild			Gautheron et al. (1994)
1-Nitropropane	108-03-2	liquid	100%	n.p.	12							7			Mild			Gautheron et al. (1994)
1-Phenyl-3-pyrazolidone	92-43-3	solid	20%	n.p.	1							7			Mild	Mild	Cat III	Gautheron et al. (1994)
1-Phenyl-3-pyrazolidone	92-43-3	solid	20%	n.p.	2							12			Mild			Gautheron et al. (1994)
1-Phenyl-3-pyrazolidone	92-43-3	solid	20%	n.p.	3							15			Mild			Gautheron et al. (1994)
1-Phenyl-3-pyrazolidone	92-43-3	solid	20%	n.p.	4							9			Mild			Gautheron et al. (1994)
1-Phenyl-3-pyrazolidone	92-43-3	solid	20%	n.p.	5							28			Moderate			Gautheron et al. (1994)
1-Phenyl-3-pyrazolidone	92-43-3	solid	20%	n.p.	6							6			Mild			Gautheron et al. (1994)
1-Phenyl-3-pyrazolidone	92-43-3	solid	20%	n.p.	7							6			Mild			Gautheron et al. (1994)
1-Phenyl-3-pyrazolidone	92-43-3	solid	20%	n.p.	8							16			Mild			Gautheron et al. (1994)
1-Phenyl-3-pyrazolidone	92-43-3	solid	20%	n.p.	9							13			Mild			Gautheron et al. (1994)
1-Phenyl-3-pyrazolidone	92-43-3	solid	20%	n.p.	10							15			Mild			Gautheron et al. (1994)
1-Phenyl-3-pyrazolidone	92-43-3	solid	20%	n.p.	11							13			Mild			Gautheron et al. (1994)
1-Phenyl-3-pyrazolidone	92-43-3	solid	20%	n.p.	12							15			Mild			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
2,4-Dichloro-5-sulfamoylbenzoic acid	2736-23-4	solid	20%	n.p.	1							23			Mild	Mild	Cat III	Gautheron et al. (1994)
2,4-Dichloro-5-sulfamoylbenzoic acid	2736-23-4	solid	20%	n.p.	2							23			Mild			Gautheron et al. (1994)
2,4-Dichloro-5-sulfamoylbenzoic acid	2736-23-4	solid	20%	n.p.	3							18			Mild			Gautheron et al. (1994)
2,4-Dichloro-5-sulfamoylbenzoic acid	2736-23-4	solid	20%	n.p.	4							28			Moderate			Gautheron et al. (1994)
2,4-Dichloro-5-sulfamoylbenzoic acid	2736-23-4	solid	20%	n.p.	5							16			Mild			Gautheron et al. (1994)
2,4-Dichloro-5-sulfamoylbenzoic acid	2736-23-4	solid	20%	n.p.	6							31			Moderate			Gautheron et al. (1994)
2,4-Dichloro-5-sulfamoylbenzoic acid	2736-23-4	solid	20%	n.p.	7							18			Mild			Gautheron et al. (1994)
2,4-Dichloro-5-sulfamoylbenzoic acid	2736-23-4	solid	20%	n.p.	8							71			Severe			Gautheron et al. (1994)
2,4-Dichloro-5-sulfamoylbenzoic acid	2736-23-4	solid	20%	n.p.	9							19			Mild			Gautheron et al. (1994)
2,4-Dichloro-5-sulfamoylbenzoic acid	2736-23-4	solid	20%	n.p.	10							20			Mild			Gautheron et al. (1994)
2,4-Dichloro-5-sulfamoylbenzoic acid	2736-23-4	solid	20%	n.p.	11							34			Moderate			Gautheron et al. (1994)
2,4-Dichloro-5-sulfamoylbenzoic acid	2736-23-4	solid	20%	n.p.	12							14			Mild			Gautheron et al. (1994)
2,4-Pentanedione	123-54-6	liquid	100%	n.p.	1							61			Severe	Severe	Cat II	Gautheron et al. (1994)
2,4-Pentanedione	123-54-6	liquid	100%	n.p.	2							79			Severe			Gautheron et al. (1994)
2,4-Pentanedione	123-54-6	liquid	100%	n.p.	3							75			Severe			Gautheron et al. (1994)
2,4-Pentanedione	123-54-6	liquid	100%	n.p.	4							34			Moderate			Gautheron et al. (1994)
2,4-Pentanedione	123-54-6	liquid	100%	n.p.	5							70			Severe			Gautheron et al. (1994)
2,4-Pentanedione	123-54-6	liquid	100%	n.p.	6							46			Moderate			Gautheron et al. (1994)
2,4-Pentanedione	123-54-6	liquid	100%	n.p.	7							54			Moderate			Gautheron et al. (1994)
2,4-Pentanedione	123-54-6	liquid	100%	n.p.	8							44			Moderate			Gautheron et al. (1994)
2,4-Pentanedione	123-54-6	liquid	100%	n.p.	9							50			Moderate			Gautheron et al. (1994)
2,4-Pentanedione	123-54-6	liquid	100%	n.p.	10							67			Severe			Gautheron et al. (1994)
2,4-Pentanedione	123-54-6	liquid	100%	n.p.	11							62			Severe			Gautheron et al. (1994)
2,4-Pentanedione	123-54-6	liquid	100%	n.p.	12							76			Severe			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
2-Aminophenol	95-55-6	solid	20%	n.p.	1							7			Mild	Mild	Cat III	Gautheron et al. (1994)
2-Aminophenol	95-55-6	solid	20%	n.p.	2							5			Mild			Gautheron et al. (1994)
2-Aminophenol	95-55-6	solid	20%	n.p.	3							3			Not Labeled			Gautheron et al. (1994)
2-Aminophenol	95-55-6	solid	20%	n.p.	4							5			Mild			Gautheron et al. (1994)
2-Aminophenol	95-55-6	solid	20%	n.p.	5							6			Mild			Gautheron et al. (1994)
2-Aminophenol	95-55-6	solid	20%	n.p.	6							7			Mild			Gautheron et al. (1994)
2-Aminophenol	95-55-6	solid	20%	n.p.	7							5			Mild			Gautheron et al. (1994)
2-Aminophenol	95-55-6	solid	20%	n.p.	8							6			Mild			Gautheron et al. (1994)
2-Aminophenol	95-55-6	solid	20%	n.p.	9							13			Mild			Gautheron et al. (1994)
2-Aminophenol	95-55-6	solid	20%	n.p.	10							11			Mild			Gautheron et al. (1994)
2-Aminophenol	95-55-6	solid	20%	n.p.	11							5			Mild			Gautheron et al. (1994)
2-Aminophenol	95-55-6	solid	20%	n.p.	12							11			Mild			Gautheron et al. (1994)
2-Ethoxyethanol	110-80-5	liquid	100%	n.p.	1							99			Severe	Severe	Cat I	Gautheron et al. (1994)
2-Ethoxyethanol	110-80-5	liquid	100%	n.p.	2							100			Severe			Gautheron et al. (1994)
2-Ethoxyethanol	110-80-5	liquid	100%	n.p.	3							128			Severe			Gautheron et al. (1994)
2-Ethoxyethanol	110-80-5	liquid	100%	n.p.	4							75			Severe			Gautheron et al. (1994)
2-Ethoxyethanol	110-80-5	liquid	100%	n.p.	5							75			Severe			Gautheron et al. (1994)
2-Ethoxyethanol	110-80-5	liquid	100%	n.p.	6							85			Severe			Gautheron et al. (1994)
2-Ethoxyethanol	110-80-5	liquid	100%	n.p.	7							94			Severe			Gautheron et al. (1994)
2-Ethoxyethanol	110-80-5	liquid	100%	n.p.	8							93			Severe			Gautheron et al. (1994)
2-Ethoxyethanol	110-80-5	liquid	100%	n.p.	9							84			Severe			Gautheron et al. (1994)
2-Ethoxyethanol	110-80-5	liquid	100%	n.p.	10							75			Severe			Gautheron et al. (1994)
2-Ethoxyethanol	110-80-5	liquid	100%	n.p.	11							101			Severe			Gautheron et al. (1994)
2-Ethoxyethanol	110-80-5	liquid	100%	n.p.	12							86			Severe			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
2-Mercaptopyrimidine	1450-85-7	solid	20%	n.p.	1							0			Not Labeled	Not Labeled	Cat IV	Gautheron et al. (1994)
2-Mercaptopyrimidine	1450-85-7	solid	20%	n.p.	2							-1			Not Labeled			Gautheron et al. (1994)
2-Mercaptopyrimidine	1450-85-7	solid	20%	n.p.	3							-1			Not Labeled			Gautheron et al. (1994)
2-Mercaptopyrimidine	1450-85-7	solid	20%	n.p.	4							1			Not Labeled			Gautheron et al. (1994)
2-Mercaptopyrimidine	1450-85-7	solid	20%	n.p.	5							0			Not Labeled			Gautheron et al. (1994)
2-Mercaptopyrimidine	1450-85-7	solid	20%	n.p.	6							1			Not Labeled			Gautheron et al. (1994)
2-Mercaptopyrimidine	1450-85-7	solid	20%	n.p.	7							0			Not Labeled			Gautheron et al. (1994)
2-Mercaptopyrimidine	1450-85-7	solid	20%	n.p.	8							-8			Not Labeled			Gautheron et al. (1994)
2-Mercaptopyrimidine	1450-85-7	solid	20%	n.p.	9							0			Not Labeled			Gautheron et al. (1994)
2-Mercaptopyrimidine	1450-85-7	solid	20%	n.p.	10							0			Not Labeled			Gautheron et al. (1994)
2-Mercaptopyrimidine	1450-85-7	solid	20%	n.p.	11							-4			Not Labeled			Gautheron et al. (1994)
2-Mercaptopyrimidine	1450-85-7	solid	20%	n.p.	12							-3			Not Labeled			Gautheron et al. (1994)
2-Methoxyethanol	109-86-4	liquid	100%	n.p.	1							61			Severe	Severe	Cat II	Gautheron et al. (1994)
2-Methoxyethanol	109-86-4	liquid	100%	n.p.	2							69			Severe			Gautheron et al. (1994)
2-Methoxyethanol	109-86-4	liquid	100%	n.p.	3							66			Severe			Gautheron et al. (1994)
2-Methoxyethanol	109-86-4	liquid	100%	n.p.	4							47			Moderate			Gautheron et al. (1994)
2-Methoxyethanol	109-86-4	liquid	100%	n.p.	5							48			Moderate			Gautheron et al. (1994)
2-Methoxyethanol	109-86-4	liquid	100%	n.p.	6							62			Severe			Gautheron et al. (1994)
2-Methoxyethanol	109-86-4	liquid	100%	n.p.	7							65			Severe			Gautheron et al. (1994)
2-Methoxyethanol	109-86-4	liquid	100%	n.p.	8							62			Severe			Gautheron et al. (1994)
2-Methoxyethanol	109-86-4	liquid	100%	n.p.	9							57			Severe			Gautheron et al. (1994)
2-Methoxyethanol	109-86-4	liquid	100%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
2-Methoxyethanol	109-86-4	liquid	100%	n.p.	11							74			Severe			Gautheron et al. (1994)
2-Methoxyethanol	109-86-4	liquid	100%	n.p.	12							88			Severe			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
3-Glycidoxy-propyltrimethoxysilane	2530-83-8	liquid	100%	n.p.	1							18			Mild	Mild	Cat III	Gautheron et al. (1994)
3-Glycidoxy-propyltrimethoxysilane	2530-83-8	liquid	100%	n.p.	2							24			Mild			Gautheron et al. (1994)
3-Glycidoxy-propyltrimethoxysilane	2530-83-8	liquid	100%	n.p.	3							25			Mild			Gautheron et al. (1994)
3-Glycidoxy-propyltrimethoxysilane	2530-83-8	liquid	100%	n.p.	4							14			Mild			Gautheron et al. (1994)
3-Glycidoxy-propyltrimethoxysilane	2530-83-8	liquid	100%	n.p.	5							13			Mild			Gautheron et al. (1994)
3-Glycidoxy-propyltrimethoxysilane	2530-83-8	liquid	100%	n.p.	6							6			Mild			Gautheron et al. (1994)
3-Glycidoxy-propyltrimethoxysilane	2530-83-8	liquid	100%	n.p.	7							15			Mild			Gautheron et al. (1994)
3-Glycidoxy-propyltrimethoxysilane	2530-83-8	liquid	100%	n.p.	8							18			Mild			Gautheron et al. (1994)
3-Glycidoxy-propyltrimethoxysilane	2530-83-8	liquid	100%	n.p.	9							18			Mild			Gautheron et al. (1994)
3-Glycidoxy-propyltrimethoxysilane	2530-83-8	liquid	100%	n.p.	10							4			Mild			Gautheron et al. (1994)
3-Glycidoxy-propyltrimethoxysilane	2530-83-8	liquid	100%	n.p.	11							23			Mild			Gautheron et al. (1994)
3-Glycidoxypropyltrimethoxysilan	2530-83-8	liquid	100%	n.p.	12							21			Mild			Gautheron et al. (1994)
Allyl alcohol	107-18-6	liquid	100%	n.p.	1							156			Severe	Severe	Cat I	Gautheron et al. (1994)
Allyl alcohol	107-18-6	liquid	100%	n.p.	2							138			Severe			Gautheron et al. (1994)
Allyl alcohol	107-18-6	liquid	100%	n.p.	3							232			Severe			Gautheron et al. (1994)
Allyl alcohol	107-18-6	liquid	100%	n.p.	4							156			Severe			Gautheron et al. (1994)
Allyl alcohol	107-18-6	liquid	100%	n.p.	5							132			Severe			Gautheron et al. (1994)
Allyl alcohol	107-18-6	liquid	100%	n.p.	6							191			Severe			Gautheron et al. (1994)
Allyl alcohol	107-18-6	liquid	100%	n.p.	7							190			Severe			Gautheron et al. (1994)
Allyl alcohol	107-18-6	liquid	100%	n.p.	8							166			Severe			Gautheron et al. (1994)
Allyl alcohol	107-18-6	liquid	100%	n.p.	9							123			Severe			Gautheron et al. (1994)
Allyl alcohol	107-18-6	liquid	100%	n.p.	10							101			Severe			Gautheron et al. (1994)
Allyl alcohol	107-18-6	liquid	100%	n.p.	11							200			Severe			Gautheron et al. (1994)
Allyl alcohol	107-18-6	liquid	100%	n.p.	12							90			Severe			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Aluminum hydroxide	21645-51-2	solid	20%	n.p.	1							5			Mild	Mild	Cat III	Gautheron et al. (1994)
Aluminum hydroxide	21645-51-2	solid	20%	n.p.	2							4			Mild			Gautheron et al. (1994)
Aluminum hydroxide	21645-51-2	solid	20%	n.p.	3							10			Mild			Gautheron et al. (1994)
Aluminum hydroxide	21645-51-2	solid	20%	n.p.	4							3			Not Labeled			Gautheron et al. (1994)
Aluminum hydroxide	21645-51-2	solid	20%	n.p.	5							5			Mild			Gautheron et al. (1994)
Aluminum hydroxide	21645-51-2	solid	20%	n.p.	6							28			Moderate			Gautheron et al. (1994)
Aluminum hydroxide	21645-51-2	solid	20%	n.p.	7							2			Not Labeled			Gautheron et al. (1994)
Aluminum hydroxide	21645-51-2	solid	20%	n.p.	8							4			Mild			Gautheron et al. (1994)
Aluminum hydroxide	21645-51-2	solid	20%	n.p.	9							10			Mild			Gautheron et al. (1994)
Aluminum hydroxide	21645-51-2	solid	20%	n.p.	10							6			Mild			Gautheron et al. (1994)
Aluminum hydroxide	21645-51-2	solid	20%	n.p.	11							2			Not Labeled			Gautheron et al. (1994)
Aluminum hydroxide	21645-51-2	solid	20%	n.p.	12							2			Not Labeled			Gautheron et al. (1994)
Anthracene	120-12-7	solid	20%	n.p.	1							-2			Not Labeled	Not Labeled	Cat IV	Gautheron et al. (1994)
Anthracene	120-12-7	solid	20%	n.p.	2							1			Not Labeled			Gautheron et al. (1994)
Anthracene	120-12-7	solid	20%	n.p.	3							-3			Not Labeled			Gautheron et al. (1994)
Anthracene	120-12-7	solid	20%	n.p.	4							0			Not Labeled			Gautheron et al. (1994)
Anthracene	120-12-7	solid	20%	n.p.	5							0			Not Labeled			Gautheron et al. (1994)
Anthracene	120-12-7	solid	20%	n.p.	6							-1			Not Labeled			Gautheron et al. (1994)
Anthracene	120-12-7	solid	20%	n.p.	7							0			Not Labeled			Gautheron et al. (1994)
Anthracene	120-12-7	solid	20%	n.p.	8							0			Not Labeled			Gautheron et al. (1994)
Anthracene	120-12-7	solid	20%	n.p.	9							1			Not Labeled			Gautheron et al. (1994)
Anthracene	120-12-7	solid	20%	n.p.	10							0			Not Labeled			Gautheron et al. (1994)
Anthracene	120-12-7	solid	20%	n.p.	11							-2			Not Labeled			Gautheron et al. (1994)
Anthracene	120-12-7	solid	20%	n.p.	12							2			Not Labeled			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Benzethonium chloride	121-54-0	surfactant	10%	n.p.	1							128			Severe	Severe	Cat I	Gautheron et al. (1994)
Benzethonium chloride	121-54-0	surfactant	10%	n.p.	2							124			Severe			Gautheron et al. (1994)
Benzethonium chloride	121-54-0	surfactant	10%	n.p.	3							163			Severe			Gautheron et al. (1994)
Benzethonium chloride	121-54-0	surfactant	10%	n.p.	4							106			Severe			Gautheron et al. (1994)
Benzethonium chloride	121-54-0	surfactant	10%	n.p.	5							128			Severe			Gautheron et al. (1994)
Benzethonium chloride	121-54-0	surfactant	10%	n.p.	6							129			Severe			Gautheron et al. (1994)
Benzethonium chloride	121-54-0	surfactant	10%	n.p.	7							142			Severe			Gautheron et al. (1994)
Benzethonium chloride	121-54-0	surfactant	10%	n.p.	8							129			Severe			Gautheron et al. (1994)
Benzethonium chloride	121-54-0	surfactant	10%	n.p.	9							166			Severe			Gautheron et al. (1994)
Benzethonium chloride	121-54-0	surfactant	10%	n.p.	10							no data			n.a.[13]			Gautheron et al. (1994)
Benzethonium chloride	121-54-0	surfactant	10%	n.p.	11							142			Severe			Gautheron et al. (1994)
Benzethonium chloride	121-54-0	surfactant	10%	n.p.	12							116			Severe			Gautheron et al. (1994)
Betaine monohydrate	590-47-6	solid	20%	n.p.	1							4			Mild	Mild	Cat III	Gautheron et al. (1994)
Betaine monohydrate	590-47-6	solid	20%	n.p.	2							1			Not Labeled			Gautheron et al. (1994)
Betaine monohydrate	590-47-6	solid	20%	n.p.	3							0			Not Labeled			Gautheron et al. (1994)
Betaine monohydrate	590-47-6	solid	20%	n.p.	4							1			Not Labeled			Gautheron et al. (1994)
Betaine monohydrate	590-47-6	solid	20%	n.p.	5							1			Not Labeled			Gautheron et al. (1994)
Betaine monohydrate	590-47-6	solid	20%	n.p.	6							3			Not Labeled			Gautheron et al. (1994)
Betaine monohydrate	590-47-6	solid	20%	n.p.	7							1			Not Labeled			Gautheron et al. (1994)
Betaine monohydrate	590-47-6	solid	20%	n.p.	8							-10			Not Labeled			Gautheron et al. (1994)
Betaine monohydrate	590-47-6	solid	20%	n.p.	9							4			Mild			Gautheron et al. (1994)
Betaine monohydrate	590-47-6	solid	20%	n.p.	10							-1			Not Labeled			Gautheron et al. (1994)
Betaine monohydrate	590-47-6	solid	20%	n.p.	11							1			Not Labeled			Gautheron et al. (1994)
Betaine monohydrate	590-47-6	solid	20%	n.p.	12							6			Mild			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
BRIJ-35	9002-92-0	surfactant	10%	n.p.	1							0			Not Labeled	Not Labeled	Cat IV	Gautheron et al. (1994)
BRIJ-35	9002-92-0	surfactant	10%	n.p.	2							2			Not Labeled			Gautheron et al. (1994)
BRIJ-35	9002-92-0	surfactant	10%	n.p.	3							-1			Not Labeled			Gautheron et al. (1994)
BRIJ-35	9002-92-0	surfactant	10%	n.p.	4							1			Not Labeled			Gautheron et al. (1994)
BRIJ-35	9002-92-0	surfactant	10%	n.p.	5							0			Not Labeled			Gautheron et al. (1994)
BRIJ-35	9002-92-0	surfactant	10%	n.p.	6							0			Not Labeled			Gautheron et al. (1994)
BRIJ-35	9002-92-0	surfactant	10%	n.p.	7							0			Not Labeled			Gautheron et al. (1994)
BRIJ-35	9002-92-0	surfactant	10%	n.p.	8							0			Not Labeled			Gautheron et al. (1994)
BRIJ-35	9002-92-0	surfactant	10%	n.p.	9							1			Not Labeled			Gautheron et al. (1994)
BRIJ-35	9002-92-0	surfactant	10%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
BRIJ-35	9002-92-0	surfactant	10%	n.p.	11							-2			Not Labeled			Gautheron et al. (1994)
BRIJ-35	9002-92-0	surfactant	10%	n.p.	12							-2			Not Labeled			Gautheron et al. (1994)
Butyrolactone	96-48-0	liquid	100%	n.p.	1							48			Moderate	Moderate	Cat II	Gautheron et al. (1994)
Butyrolactone	96-48-0	liquid	100%	n.p.	2							44			Moderate			Gautheron et al. (1994)
Butyrolactone	96-48-0	liquid	100%	n.p.	3							64			Severe			Gautheron et al. (1994)
Butyrolactone	96-48-0	liquid	100%	n.p.	4							35			Moderate			Gautheron et al. (1994)
Butyrolactone	96-48-0	liquid	100%	n.p.	5							35			Moderate			Gautheron et al. (1994)
Butyrolactone	96-48-0	liquid	100%	n.p.	6							30			Moderate			Gautheron et al. (1994)
Butyrolactone	96-48-0	liquid	100%	n.p.	7							80			Severe			Gautheron et al. (1994)
Butyrolactone	96-48-0	liquid	100%	n.p.	8							32			Moderate			Gautheron et al. (1994)
Butyrolactone	96-48-0	liquid	100%	n.p.	9							42			Moderate			Gautheron et al. (1994)
Butyrolactone	96-48-0	liquid	100%	n.p.	10							53			Moderate			Gautheron et al. (1994)
Butyrolactone	96-48-0	liquid	100%	n.p.	11							35			Moderate			Gautheron et al. (1994)
Butyrolactone	96-48-0	liquid	100%	n.p.	12							49			Moderate			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Cyclohexanone	108-94-1	liquid	100%	n.p.	1							92			Severe	Severe	Cat I	Gautheron et al. (1994)
Cyclohexanone	108-94-1	liquid	100%	n.p.	2							108			Severe			Gautheron et al. (1994)
Cyclohexanone	108-94-1	liquid	100%	n.p.	3							96			Severe			Gautheron et al. (1994)
Cyclohexanone	108-94-1	liquid	100%	n.p.	4							81			Severe			Gautheron et al. (1994)
Cyclohexanone	108-94-1	liquid	100%	n.p.	5							130			Severe			Gautheron et al. (1994)
Cyclohexanone	108-94-1	liquid	100%	n.p.	6							93			Severe			Gautheron et al. (1994)
Cyclohexanone	108-94-1	liquid	100%	n.p.	7							104			Severe			Gautheron et al. (1994)
Cyclohexanone	108-94-1	liquid	100%	n.p.	8							90			Severe			Gautheron et al. (1994)
Cyclohexanone	108-94-1	liquid	100%	n.p.	9							142			Severe			Gautheron et al. (1994)
Cyclohexanone	108-94-1	liquid	100%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Cyclohexanone	108-94-1	liquid	100%	n.p.	11							118			Severe			Gautheron et al. (1994)
Cyclohexanone	108-94-1	liquid	100%	n.p.	12							108			Severe			Gautheron et al. (1994)
Deoxycholic acid, sodium salt	302-95-4	surfactant	10%	n.p.	1							96			Severe	Severe	Cat I	Gautheron et al. (1994)
Deoxycholic acid, sodium salt	302-95-4	surfactant	10%	n.p.	2							72			Severe			Gautheron et al. (1994)
Deoxycholic acid, sodium salt	302-95-4	surfactant	10%	n.p.	3							106			Severe			Gautheron et al. (1994)
Deoxycholic acid, sodium salt	302-95-4	surfactant	10%	n.p.	4							73			Severe			Gautheron et al. (1994)
Deoxycholic acid, sodium salt	302-95-4	surfactant	10%	n.p.	5							119			Severe			Gautheron et al. (1994)
Deoxycholic acid, sodium salt	302-95-4	surfactant	10%	n.p.	6							103			Severe			Gautheron et al. (1994)
Deoxycholic acid, sodium salt	302-95-4	surfactant	10%	n.p.	7							88			Severe			Gautheron et al. (1994)
Deoxycholic acid, sodium salt	302-95-4	surfactant	10%	n.p.	8							46			Moderate			Gautheron et al. (1994)
Deoxycholic acid, sodium salt	302-95-4	surfactant	10%	n.p.	9							100			Severe			Gautheron et al. (1994)
Deoxycholic acid, sodium salt	302-95-4	surfactant	10%	n.p.	10							60			Severe			Gautheron et al. (1994)
Deoxycholic acid, sodium salt	302-95-4	surfactant	10%	n.p.	11							200			Severe			Gautheron et al. (1994)
Deoxycholic acid, sodium salt	302-95-4	surfactant	10%	n.p.	12							59			Severe			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Diacetone alcohol	123-42-2	liquid	100%	n.p.	1							53			Moderate	Moderate	Cat II	Gautheron et al. (1994)
Diacetone alcohol	123-42-2	liquid	100%	n.p.	2							41			Moderate			Gautheron et al. (1994)
Diacetone alcohol	123-42-2	liquid	100%	n.p.	3							105			Severe			Gautheron et al. (1994)
Diacetone alcohol	123-42-2	liquid	100%	n.p.	4							39			Moderate			Gautheron et al. (1994)
Diacetone alcohol	123-42-2	liquid	100%	n.p.	5							42			Moderate			Gautheron et al. (1994)
Diacetone alcohol	123-42-2	liquid	100%	n.p.	6							34			Moderate			Gautheron et al. (1994)
Diacetone alcohol	123-42-2	liquid	100%	n.p.	7							49			Moderate			Gautheron et al. (1994)
Diacetone alcohol	123-42-2	liquid	100%	n.p.	8							41			Moderate			Gautheron et al. (1994)
Diacetone alcohol	123-42-2	liquid	100%	n.p.	9							92			Severe			Gautheron et al. (1994)
Diacetone alcohol	123-42-2	liquid	100%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Diacetone alcohol	123-42-2	liquid	100%	n.p.	11							36			Moderate			Gautheron et al. (1994)
Diacetone alcohol	123-42-2	liquid	100%	n.p.	12							56			Severe			Gautheron et al. (1994)
Dibenzoyl-L-tartaric acid	2743-38-6	solid	20%	n.p.	1							104			Severe	Severe	Cat I	Gautheron et al. (1994)
Dibenzoyl-L-tartaric acid	2743-38-6	solid	20%	n.p.	2							134			Severe			Gautheron et al. (1994)
Dibenzoyl-L-tartaric acid	2743-38-6	solid	20%	n.p.	3							82			Severe			Gautheron et al. (1994)
Dibenzoyl-L-tartaric acid	2743-38-6	solid	20%	n.p.	4							118			Severe			Gautheron et al. (1994)
Dibenzoyl-L-tartaric acid	2743-38-6	solid	20%	n.p.	5							110			Severe			Gautheron et al. (1994)
Dibenzoyl-L-tartaric acid	2743-38-6	solid	20%	n.p.	6							66			Severe			Gautheron et al. (1994)
Dibenzoyl-L-tartaric acid	2743-38-6	solid	20%	n.p.	7							88			Severe			Gautheron et al. (1994)
Dibenzoyl-L-tartaric acid	2743-38-6	solid	20%	n.p.	8							193			Severe			Gautheron et al. (1994)
Dibenzoyl-L-tartaric acid	2743-38-6	solid	20%	n.p.	9							82			Severe			Gautheron et al. (1994)
Dibenzoyl-L-tartaric acid	2743-38-6	solid	20%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Dibenzoyl-L-tartaric acid	2743-38-6	solid	20%	n.p.	11							213			Severe			Gautheron et al. (1994)
Dibenzoyl-L-tartaric acid	2743-38-6	solid	20%	n.p.	12							135			Severe			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Dimethyl sulfoxide	67-68-5	liquid	100%	n.p.	1							10			Mild	Mild	Cat III	Gautheron et al. (1994)
Dimethyl sulfoxide	67-68-5	liquid	100%	n.p.	2							10			Mild			Gautheron et al. (1994)
Dimethyl sulfoxide	67-68-5	liquid	100%	n.p.	3							14			Mild			Gautheron et al. (1994)
Dimethyl sulfoxide	67-68-5	liquid	100%	n.p.	4							11			Mild			Gautheron et al. (1994)
Dimethyl sulfoxide	67-68-5	liquid	100%	n.p.	5							11			Mild			Gautheron et al. (1994)
Dimethyl sulfoxide	67-68-5	liquid	100%	n.p.	6							14			Mild			Gautheron et al. (1994)
Dimethyl sulfoxide	67-68-5	liquid	100%	n.p.	7							10			Mild			Gautheron et al. (1994)
Dimethyl sulfoxide	67-68-5	liquid	100%	n.p.	8							10			Mild			Gautheron et al. (1994)
Dimethyl sulfoxide	67-68-5	liquid	100%	n.p.	9							9			Mild			Gautheron et al. (1994)
Dimethyl sulfoxide	67-68-5	liquid	100%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Dimethyl sulfoxide	67-68-5	liquid	100%	n.p.	11							4			Mild			Gautheron et al. (1994)
Dimethyl sulfoxide	67-68-5	liquid	100%	n.p.	12							22			Mild			Gautheron et al. (1994)
Dimethylbiguanide	657-24-9	solid	20%	n.p.	1							0			Not Labeled	Not Labeled	Cat IV	Gautheron et al. (1994)
Dimethylbiguanide	657-24-9	solid	20%	n.p.	2							3			Not Labeled			Gautheron et al. (1994)
Dimethylbiguanide	657-24-9	solid	20%	n.p.	3							1			Not Labeled			Gautheron et al. (1994)
Dimethylbiguanide	657-24-9	solid	20%	n.p.	4							3			Not Labeled			Gautheron et al. (1994)
Dimethylbiguanide	657-24-9	solid	20%	n.p.	5							1			Not Labeled			Gautheron et al. (1994)
Dimethylbiguanide	657-24-9	solid	20%	n.p.	6							5			Mild			Gautheron et al. (1994)
Dimethylbiguanide	657-24-9	solid	20%	n.p.	7							3			Not Labeled			Gautheron et al. (1994)
Dimethylbiguanide	657-24-9	solid	20%	n.p.	8							1			Not Labeled			Gautheron et al. (1994)
Dimethylbiguanide	657-24-9	solid	20%	n.p.	9							2			Not Labeled			Gautheron et al. (1994)
Dimethylbiguanide	657-24-9	solid	20%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Dimethylbiguanide	657-24-9	solid	20%	n.p.	11							5			Mild			Gautheron et al. (1994)
Dimethylbiguanide	657-24-9	solid	20%	n.p.	12							8			Mild			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
DL-Glutamic acid	19285-83-7	solid	20%	n.p.	1							2			Not Labeled	Not Labeled	Cat IV	Gautheron et al. (1994)
DL-Glutamic acid	19285-83-7	solid	20%	n.p.	2							1			Not Labeled			Gautheron et al. (1994)
DL-Glutamic acid	19285-83-7	solid	20%	n.p.	3							1			Not Labeled			Gautheron et al. (1994)
DL-Glutamic acid	19285-83-7	solid	20%	n.p.	4							1			Not Labeled			Gautheron et al. (1994)
DL-Glutamic acid	19285-83-7	solid	20%	n.p.	5							0			Not Labeled			Gautheron et al. (1994)
DL-Glutamic acid	19285-83-7	solid	20%	n.p.	6							-4			Not Labeled			Gautheron et al. (1994)
DL-Glutamic acid	19285-83-7	solid	20%	n.p.	7							1			Not Labeled			Gautheron et al. (1994)
DL-Glutamic acid	19285-83-7	solid	20%	n.p.	8							4			Mild			Gautheron et al. (1994)
DL-Glutamic acid	19285-83-7	solid	20%	n.p.	9							0			Not Labeled			Gautheron et al. (1994)
DL-Glutamic acid	19285-83-7	solid	20%	n.p.	10							2			Not Labeled			Gautheron et al. (1994)
DL-Glutamic acid	19285-83-7	solid	20%	n.p.	11							0			Not Labeled			Gautheron et al. (1994)
DL-Glutamic acid	19285-83-7	solid	20%	n.p.	12							-1			Not Labeled			Gautheron et al. (1994)
Ethanol	64-17-5	liquid	100%	n.p.	1							58			Severe	Severe	Cat I	Gautheron et al. (1994)
Ethanol	64-17-5	liquid	100%	n.p.	2							67			Severe			Gautheron et al. (1994)
Ethanol	64-17-5	liquid	100%	n.p.	3							70			Severe			Gautheron et al. (1994)
Ethanol	64-17-5	liquid	100%	n.p.	4							45			Moderate			Gautheron et al. (1994)
Ethanol	64-17-5	liquid	100%	n.p.	5							60			Severe			Gautheron et al. (1994)
Ethanol	64-17-5	liquid	100%	n.p.	6							64			Severe			Gautheron et al. (1994)
Ethanol	64-17-5	liquid	100%	n.p.	7							58			Severe			Gautheron et al. (1994)
Ethanol	64-17-5	liquid	100%	n.p.	8							51			Moderate			Gautheron et al. (1994)
Ethanol	64-17-5	liquid	100%	n.p.	9	22.3	6	4.1	1.56	6	0.316	46	6	6.6	Moderate			Gautheron et al. (1994)
Ethanol	64-17-5	liquid	100%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Ethanol	64-17-5	liquid	100%	n.p.	11							104			Severe			Gautheron et al. (1994)
Ethanol	64-17-5	liquid	100%	n.p.	12							45			Moderate			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
EDTA, di-potassium salt	25102-12-9	solid	20%	n.p.	1							-1			Not Labeled	Not Labeled	Cat IV	Gautheron et al. (1994)
EDTA, di-potassium salt	25102-12-9	solid	20%	n.p.	2							0			Not Labeled			Gautheron et al. (1994)
EDTA, di-potassium salt	25102-12-9	solid	20%	n.p.	3							-8			Not Labeled			Gautheron et al. (1994)
EDTA, di-potassium salt	25102-12-9	solid	20%	n.p.	4							2			Not Labeled			Gautheron et al. (1994)
EDTA, di-potassium salt	25102-12-9	solid	20%	n.p.	5							2			Not Labeled			Gautheron et al. (1994)
EDTA, di-potassium salt	25102-12-9	solid	20%	n.p.	6							2			Not Labeled			Gautheron et al. (1994)
EDTA, di-potassium salt	25102-12-9	solid	20%	n.p.	7							1			Not Labeled			Gautheron et al. (1994)
EDTA, di-potassium salt	25102-12-9	solid	20%	n.p.	8							-6			Not Labeled			Gautheron et al. (1994)
EDTA, di-potassium salt	25102-12-9	solid	20%	n.p.	9							1			Not Labeled			Gautheron et al. (1994)
EDTA, di-potassium salt	25102-12-9	solid	20%	n.p.	10							-1			Not Labeled			Gautheron et al. (1994)
EDTA, di-potassium salt	25102-12-9	solid	20%	n.p.	11							3			Not Labeled			Gautheron et al. (1994)
EDTA, di-potassium salt	25102-12-9	solid	20%	n.p.	12							1			Not Labeled			Gautheron et al. (1994)
Ethyl acetoacetate	141-97-9	liquid	100%	n.p.	1							26			Moderate	Moderate	Cat II	Gautheron et al. (1994)
Ethyl acetoacetate	141-97-9	liquid	100%	n.p.	2							38			Moderate			Gautheron et al. (1994)
Ethyl acetoacetate	141-97-9	liquid	100%	n.p.	3							31			Moderate			Gautheron et al. (1994)
Ethyl acetoacetate	141-97-9	liquid	100%	n.p.	4							33			Moderate			Gautheron et al. (1994)
Ethyl acetoacetate	141-97-9	liquid	100%	n.p.	5							21			Moderate			Gautheron et al. (1994)
Ethyl acetoacetate	141-97-9	liquid	100%	n.p.	6							29			Moderate			Gautheron et al. (1994)
Ethyl acetoacetate	141-97-9	liquid	100%	n.p.	7							28			Moderate			Gautheron et al. (1994)
Ethyl acetoacetate	141-97-9	liquid	100%	n.p.	8							38			Moderate			Gautheron et al. (1994)
Ethyl acetoacetate	141-97-9	liquid	100%	n.p.	9							26			Moderate			Gautheron et al. (1994)
Ethyl acetoacetate	141-97-9	liquid	100%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Ethyl acetoacetate	141-97-9	liquid	100%	n.p.	11							38			Moderate			Gautheron et al. (1994)
Ethyl acetoacetate	141-97-9	liquid	100%	n.p.	12							42			Moderate			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Furan	110-00-9	liquid	100%	n.p.	1							73			Severe	Severe	Cat II	Gautheron et al. (1994)
Furan	110-00-9	liquid	100%	n.p.	2							63			Severe			Gautheron et al. (1994)
Furan	110-00-9	liquid	100%	n.p.	3							61			Severe			Gautheron et al. (1994)
Furan	110-00-9	liquid	100%	n.p.	4							65			Severe			Gautheron et al. (1994)
Furan	110-00-9	liquid	100%	n.p.	5							33			Moderate			Gautheron et al. (1994)
Furan	110-00-9	liquid	100%	n.p.	6							34			Moderate			Gautheron et al. (1994)
Furan	110-00-9	liquid	100%	n.p.	7							87			Severe			Gautheron et al. (1994)
Furan	110-00-9	liquid	100%	n.p.	8							48			Moderate			Gautheron et al. (1994)
Furan	110-00-9	liquid	100%	n.p.	9							50			Moderate			Gautheron et al. (1994)
Furan	110-00-9	liquid	100%	n.p.	10							39			Moderate			Gautheron et al. (1994)
Furan	110-00-9	liquid	100%	n.p.	11							68			Severe			Gautheron et al. (1994)
Furan	110-00-9	liquid	100%	n.p.	12							51			Moderate			Gautheron et al. (1994)
Gluconolactone	90-80-2	solid	20%	n.p.	1							63			Severe	Severe	Cat I	Gautheron et al. (1994)
Gluconolactone	90-80-2	solid	20%	n.p.	2							81			Severe			Gautheron et al. (1994)
Gluconolactone	90-80-2	solid	20%	n.p.	3							90			Severe			Gautheron et al. (1994)
Gluconolactone	90-80-2	solid	20%	n.p.	4							62			Severe			Gautheron et al. (1994)
Gluconolactone	90-80-2	solid	20%	n.p.	5							108			Severe			Gautheron et al. (1994)
Gluconolactone	90-80-2	solid	20%	n.p.	6							66			Severe			Gautheron et al. (1994)
Gluconolactone	90-80-2	solid	20%	n.p.	7							90			Severe			Gautheron et al. (1994)
Gluconolactone	90-80-2	solid	20%	n.p.	8							57			Severe			Gautheron et al. (1994)
Gluconolactone	90-80-2	solid	20%	n.p.	9							88			Severe			Gautheron et al. (1994)
Gluconolactone	90-80-2	solid	20%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Gluconolactone	90-80-2	solid	20%	n.p.	11							75			Severe			Gautheron et al. (1994)
Gluconolactone	90-80-2	solid	20%	n.p.	12							63			Severe			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	1							93			Severe	Moderate	Cat II	Gautheron et al. (1994)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	2							40			Moderate			Gautheron et al. (1994)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	3							53			Moderate			Gautheron et al. (1994)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	4							33			Moderate			Gautheron et al. (1994)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	5							91			Severe			Gautheron et al. (1994)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	6							42			Moderate			Gautheron et al. (1994)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	7							82			Severe			Gautheron et al. (1994)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	8							76			Severe			Gautheron et al. (1994)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	9							70			Severe			Gautheron et al. (1994)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	11							48			Moderate			Gautheron et al. (1994)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	12							102			Severe			Gautheron et al. (1994)
Hexane	110-54-3	liquid	100%	n.p.	1							2			Not Labeled	Not Labeled	Cat IV	Gautheron et al. (1994)
Hexane	110-54-3	liquid	100%	n.p.	2							2			Not Labeled			Gautheron et al. (1994)
Hexane	110-54-3	liquid	100%	n.p.	3							2			Not Labeled			Gautheron et al. (1994)
Hexane	110-54-3	liquid	100%	n.p.	4							0			Not Labeled			Gautheron et al. (1994)
Hexane	110-54-3	liquid	100%	n.p.	5							2			Not Labeled			Gautheron et al. (1994)
Hexane	110-54-3	liquid	100%	n.p.	6							1			Not Labeled			Gautheron et al. (1994)
Hexane	110-54-3	liquid	100%	n.p.	7							3			Not Labeled			Gautheron et al. (1994)
Hexane	110-54-3	liquid	100%	n.p.	8							1			Not Labeled			Gautheron et al. (1994)
Hexane	110-54-3	liquid	100%	n.p.	9							1			Not Labeled			Gautheron et al. (1994)
Hexane	110-54-3	liquid	100%	n.p.	10							-1			Not Labeled			Gautheron et al. (1994)
Hexane	110-54-3	liquid	100%	n.p.	11							-2			Not Labeled			Gautheron et al. (1994)
Hexane	110-54-3	liquid	100%	n.p.	12							6			Mild			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Imidazole	288-32-4	solid	20%	n.p.	1							75			Severe	Severe		Gautheron et al. (1994)
Imidazole	288-32-4	solid	20%	n.p.	2							73			Severe			Gautheron et al. (1994)
Imidazole	288-32-4	solid	20%	n.p.	3							140			Severe			Gautheron et al. (1994)
Imidazole	288-32-4	solid	20%	n.p.	4							81			Severe			Gautheron et al. (1994)
Imidazole	288-32-4	solid	20%	n.p.	5							96			Severe			Gautheron et al. (1994)
Imidazole	288-32-4	solid	20%	n.p.	6							62			Severe			Gautheron et al. (1994)
Imidazole	288-32-4	solid	20%	n.p.	7							82			Severe			Gautheron et al. (1994)
Imidazole	288-32-4	solid	20%	n.p.	8							122			Severe			Gautheron et al. (1994)
Imidazole	288-32-4	solid	20%	n.p.	9							64			Severe			Gautheron et al. (1994)
Imidazole	288-32-4	solid	20%	n.p.	10							81			Severe			Gautheron et al. (1994)
Imidazole	288-32-4	solid	20%	n.p.	11							114			Severe			Gautheron et al. (1994)
Imidazole	288-32-4	solid	20%	n.p.	12							65			Severe			Gautheron et al. (1994)
Iminodibenzyl	494-19-9	solid	20%	n.p.	1							0			Not Labeled	Not Labeled	Cat IV	Gautheron et al. (1994)
Iminodibenzyl	494-19-9	solid	20%	n.p.	2							1			Not Labeled			Gautheron et al. (1994)
Iminodibenzyl	494-19-9	solid	20%	n.p.	3							6			Mild			Gautheron et al. (1994)
Iminodibenzyl	494-19-9	solid	20%	n.p.	4							0			Not Labeled			Gautheron et al. (1994)
Iminodibenzyl	494-19-9	solid	20%	n.p.	5							4			Mild			Gautheron et al. (1994)
Iminodibenzyl	494-19-9	solid	20%	n.p.	6							0			Not Labeled			Gautheron et al. (1994)
Iminodibenzyl	494-19-9	solid	20%	n.p.	7							1			Not Labeled			Gautheron et al. (1994)
Iminodibenzyl	494-19-9	solid	20%	n.p.	8							12			Mild			Gautheron et al. (1994)
Iminodibenzyl	494-19-9	solid	20%	n.p.	9							0			Not Labeled			Gautheron et al. (1994)
Iminodibenzyl	494-19-9	solid	20%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Iminodibenzyl	494-19-9	solid	20%	n.p.	11							6			Mild			Gautheron et al. (1994)
Iminodibenzyl	494-19-9	solid	20%	n.p.	12							-4			Not Labeled			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Laurylsulfobetaine	14933-08-5	surfactant	10%	n.p.	1							81			Severe	Severe	Cat I	Gautheron et al. (1994)
Laurylsulfobetaine	14933-08-5	surfactant	10%	n.p.	2							82			Severe			Gautheron et al. (1994)
Laurylsulfobetaine	14933-08-5	surfactant	10%	n.p.	3							103			Severe			Gautheron et al. (1994)
Laurylsulfobetaine	14933-08-5	surfactant	10%	n.p.	4							76			Severe			Gautheron et al. (1994)
Laurylsulfobetaine	14933-08-5	surfactant	10%	n.p.	5							92			Severe			Gautheron et al. (1994)
Laurylsulfobetaine	14933-08-5	surfactant	10%	n.p.	6							68			Severe			Gautheron et al. (1994)
Laurylsulfobetaine	14933-08-5	surfactant	10%	n.p.	7							90			Severe			Gautheron et al. (1994)
Laurylsulfobetaine	14933-08-5	surfactant	10%	n.p.	8							62			Severe			Gautheron et al. (1994)
Laurylsulfobetaine	14933-08-5	surfactant	10%	n.p.	9							102			Severe			Gautheron et al. (1994)
Laurylsulfobetaine	14933-08-5	surfactant	10%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Laurylsulfobetaine	14933-08-5	surfactant	10%	n.p.	11							76			Severe			Gautheron et al. (1994)
Laurylsulfobetaine	14933-08-5	surfactant	10%	n.p.	12							55			Moderate			Gautheron et al. (1994)
Magnesium carbonate	56378-72-4	solid	20%	n.p.	1							3			Not Labeled	Not Labeled	Cat IV	Gautheron et al. (1994)
Magnesium carbonate	56378-72-4	solid	20%	n.p.	2							6			Mild			Gautheron et al. (1994)
Magnesium carbonate	56378-72-4	solid	20%	n.p.	3							3			Not Labeled			Gautheron et al. (1994)
Magnesium carbonate	56378-72-4	solid	20%	n.p.	4							3			Not Labeled			Gautheron et al. (1994)
Magnesium carbonate	56378-72-4	solid	20%	n.p.	5							0			Not Labeled			Gautheron et al. (1994)
Magnesium carbonate	56378-72-4	solid	20%	n.p.	6							1			Not Labeled			Gautheron et al. (1994)
Magnesium carbonate	56378-72-4	solid	20%	n.p.	7							7			Mild			Gautheron et al. (1994)
Magnesium carbonate	56378-72-4	solid	20%	n.p.	8							3			Not Labeled			Gautheron et al. (1994)
Magnesium carbonate	56378-72-4	solid	20%	n.p.	9							1			Not Labeled			Gautheron et al. (1994)
Magnesium carbonate	56378-72-4	solid	20%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Magnesium carbonate	56378-72-4	solid	20%	n.p.	11							0			Not Labeled			Gautheron et al. (1994)
Magnesium carbonate	56378-72-4	solid	20%	n.p.	12							6			Mild			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Methanol	67-56-1	liquid	100%	n.p.	1							88			Severe	Severe	Cat I	Gautheron et al. (1994)
Methanol	67-56-1	liquid	100%	n.p.	2							88			Severe			Gautheron et al. (1994)
Methanol	67-56-1	liquid	100%	n.p.	3							54			Moderate			Gautheron et al. (1994)
Methanol	67-56-1	liquid	100%	n.p.	4							71			Severe			Gautheron et al. (1994)
Methanol	67-56-1	liquid	100%	n.p.	5							81			Severe			Gautheron et al. (1994)
Methanol	67-56-1	liquid	100%	n.p.	6							108			Severe			Gautheron et al. (1994)
Methanol	67-56-1	liquid	100%	n.p.	7							37			Moderate			Gautheron et al. (1994)
Methanol	67-56-1	liquid	100%	n.p.	8							19			Mild			Gautheron et al. (1994)
Methanol	67-56-1	liquid	100%	n.p.	9							99			Severe			Gautheron et al. (1994)
Methanol	67-56-1	liquid	100%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Methanol	67-56-1	liquid	100%	n.p.	11							179			Severe			Gautheron et al. (1994)
Methanol	67-56-1	liquid	100%	n.p.	12							102			Severe			Gautheron et al. (1994)
Methylisobutyl ketone	108-10-1	liquid	100%	n.p.	1							22			Mild	Mild		Gautheron et al. (1994)
Methylisobutyl ketone	108-10-1	liquid	100%	n.p.	2							25			Mild			Gautheron et al. (1994)
Methylisobutyl ketone	108-10-1	liquid	100%	n.p.	3							27			Moderate			Gautheron et al. (1994)
Methylisobutyl ketone	108-10-1	liquid	100%	n.p.	4							19			Mild			Gautheron et al. (1994)
Methylisobutyl ketone	108-10-1	liquid	100%	n.p.	5							21			Mild			Gautheron et al. (1994)
Methylisobutyl ketone	108-10-1	liquid	100%	n.p.	6							23			Mild			Gautheron et al. (1994)
Methylisobutyl ketone	108-10-1	liquid	100%	n.p.	7							16			Mild			Gautheron et al. (1994)
Methylisobutyl ketone	108-10-1	liquid	100%	n.p.	8							16			Mild			Gautheron et al. (1994)
Methylisobutyl ketone	108-10-1	liquid	100%	n.p.	9							19			Mild			Gautheron et al. (1994)
Methylisobutyl ketone	108-10-1	liquid	100%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Methylisobutyl ketone	108-10-1	liquid	100%	n.p.	11							20			Mild			Gautheron et al. (1994)
Methylisobutyl ketone	108-10-1	liquid	100%	n.p.	12							11			Mild			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
MYRJ-45	-	surfactant	10%	n.p.	1							2			Not Labeled	Not Labeled	Cat IV	Gautheron et al. (1994)
MYRJ-45	-	surfactant	10%	n.p.	2							1			Not Labeled			Gautheron et al. (1994)
MYRJ-45	-	surfactant	10%	n.p.	3							0			Not Labeled			Gautheron et al. (1994)
MYRJ-45	-	surfactant	10%	n.p.	4							1			Not Labeled			Gautheron et al. (1994)
MYRJ-45	-	surfactant	10%	n.p.	5							0			Not Labeled			Gautheron et al. (1994)
MYRJ-45	-	surfactant	10%	n.p.	6							0			Not Labeled			Gautheron et al. (1994)
MYRJ-45	-	surfactant	10%	n.p.	7							1			Not Labeled			Gautheron et al. (1994)
MYRJ-45	-	surfactant	10%	n.p.	8							-4			Not Labeled			Gautheron et al. (1994)
MYRJ-45	-	surfactant	10%	n.p.	9							1			Not Labeled			Gautheron et al. (1994)
MYRJ-45	-	surfactant	10%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
MYRJ-45	-	surfactant	10%	n.p.	11							-3			Not Labeled			Gautheron et al. (1994)
MYRJ-45	-	surfactant	10%	n.p.	12							-1			Not Labeled			Gautheron et al. (1994)
N-Lauroylsarcosine, sodium salt	7631-98-3	surfactant	10%	n.p.	1							53			Moderate	Moderate	Cat II	Gautheron et al. (1994)
N-Lauroylsarcosine, sodium salt	7631-98-3	surfactant	10%	n.p.	2							50			Moderate			Gautheron et al. (1994)
N-Lauroylsarcosine, sodium salt	7631-98-3	surfactant	10%	n.p.	3							48			Moderate			Gautheron et al. (1994)
N-Lauroylsarcosine, sodium salt	7631-98-3	surfactant	10%	n.p.	4							28			Moderate			Gautheron et al. (1994)
N-Lauroylsarcosine, sodium salt	7631-98-3	surfactant	10%	n.p.	5							45			Moderate			Gautheron et al. (1994)
N-Lauroylsarcosine, sodium salt	7631-98-3	surfactant	10%	n.p.	6							35			Moderate			Gautheron et al. (1994)
N-Lauroylsarcosine, sodium salt	7631-98-3	surfactant	10%	n.p.	7							48			Moderate			Gautheron et al. (1994)
N-Lauroylsarcosine, sodium salt	7631-98-3	surfactant	10%	n.p.	8							43			Moderate			Gautheron et al. (1994)
N-Lauroylsarcosine, sodium salt	7631-98-3	surfactant	10%	n.p.	9							63			Severe			Gautheron et al. (1994)
N-Lauroylsarcosine, sodium salt	7631-98-3	surfactant	10%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
N-Lauroylsarcosine, sodium salt	7631-98-3	surfactant	10%	n.p.	11							89			Severe			Gautheron et al. (1994)
N-Lauroylsarcosine, sodium salt	7631-98-3	surfactant	10%	n.p.	12							48			Moderate			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Octanol	111-87-5	liquid	100%	n.p.	1							65			Severe	Moderate	Cat II	Gautheron et al. (1994)
Octanol	111-87-5	liquid	100%	n.p.	2							33			Moderate			Gautheron et al. (1994)
Octanol	111-87-5	liquid	100%	n.p.	3							42			Moderate			Gautheron et al. (1994)
Octanol	111-87-5	liquid	100%	n.p.	4							49			Moderate			Gautheron et al. (1994)
Octanol	111-87-5	liquid	100%	n.p.	5							66			Severe			Gautheron et al. (1994)
Octanol	111-87-5	liquid	100%	n.p.	6							48			Moderate			Gautheron et al. (1994)
Octanol	111-87-5	liquid	100%	n.p.	7							37			Moderate			Gautheron et al. (1994)
Octanol	111-87-5	liquid	100%	n.p.	8							25			Mild			Gautheron et al. (1994)
Octanol	111-87-5	liquid	100%	n.p.	9							61			Severe			Gautheron et al. (1994)
Octanol	111-87-5	liquid	100%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Octanol	111-87-5	liquid	100%	n.p.	11							31			Moderate			Gautheron et al. (1994)
Octanol	111-87-5	liquid	100%	n.p.	12							64			Severe			Gautheron et al. (1994)
Petroleum ether	8032-32-4	liquid	100%	n.p.	1							8			Mild	Mild	Cat III	Gautheron et al. (1994)
Petroleum ether	8032-32-4	liquid	100%	n.p.	2							13			Mild			Gautheron et al. (1994)
Petroleum ether	8032-32-4	liquid	100%	n.p.	3							11			Mild			Gautheron et al. (1994)
Petroleum ether	8032-32-4	liquid	100%	n.p.	4							1			Not Labeled			Gautheron et al. (1994)
Petroleum ether	8032-32-4	liquid	100%	n.p.	5							2			Not Labeled			Gautheron et al. (1994)
Petroleum ether	8032-32-4	liquid	100%	n.p.	6							5			Mild			Gautheron et al. (1994)
Petroleum ether	8032-32-4	liquid	100%	n.p.	7							7			Mild			Gautheron et al. (1994)
Petroleum ether	8032-32-4	liquid	100%	n.p.	8							0			Not Labeled			Gautheron et al. (1994)
Petroleum ether	8032-32-4	liquid	100%	n.p.	9							2			Not Labeled			Gautheron et al. (1994)
Petroleum ether	8032-32-4	liquid	100%	n.p.	10							3			Not Labeled			Gautheron et al. (1994)
Petroleum ether	8032-32-4	liquid	100%	n.p.	11							5			Mild			Gautheron et al. (1994)
Petroleum ether	8032-32-4	liquid	100%	n.p.	12							9			Mild			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Phenylbutazone	50-33-9	solid	20%	n.p.	1							0			Not Labeled	Not Labeled	Cat IV	Gautheron et al. (1994)
Phenylbutazone	50-33-9	solid	20%	n.p.	2							1			Not Labeled			Gautheron et al. (1994)
Phenylbutazone	50-33-9	solid	20%	n.p.	3							1			Not Labeled			Gautheron et al. (1994)
Phenylbutazone	50-33-9	solid	20%	n.p.	4							0			Not Labeled			Gautheron et al. (1994)
Phenylbutazone	50-33-9	solid	20%	n.p.	5							0			Not Labeled			Gautheron et al. (1994)
Phenylbutazone	50-33-9	solid	20%	n.p.	6							1			Not Labeled			Gautheron et al. (1994)
Phenylbutazone	50-33-9	solid	20%	n.p.	7							0			Not Labeled			Gautheron et al. (1994)
Phenylbutazone	50-33-9	solid	20%	n.p.	8							-6			Not Labeled			Gautheron et al. (1994)
Phenylbutazone	50-33-9	solid	20%	n.p.	9							1			Not Labeled			Gautheron et al. (1994)
Phenylbutazone	50-33-9	solid	20%	n.p.	10							1			Not Labeled			Gautheron et al. (1994)
Phenylbutazone	50-33-9	solid	20%	n.p.	11							-3			Not Labeled			Gautheron et al. (1994)
Phenylbutazone	50-33-9	solid	20%	n.p.	12							2			Not Labeled			Gautheron et al. (1994)
Promethazine hydrochloride	58-33-3	solid	20%	n.p.	1							117			Severe	Severe	Cat I	Gautheron et al. (1994)
Promethazine hydrochloride	58-33-3	solid	20%	n.p.	2							156			Severe			Gautheron et al. (1994)
Promethazine hydrochloride	58-33-3	solid	20%	n.p.	3							109			Severe			Gautheron et al. (1994)
Promethazine hydrochloride	58-33-3	solid	20%	n.p.	4							111			Severe			Gautheron et al. (1994)
Promethazine hydrochloride	58-33-3	solid	20%	n.p.	5							164			Severe			Gautheron et al. (1994)
Promethazine hydrochloride	58-33-3	solid	20%	n.p.	6							174			Severe			Gautheron et al. (1994)
Promethazine hydrochloride	58-33-3	solid	20%	n.p.	7							103			Severe			Gautheron et al. (1994)
Promethazine hydrochloride	58-33-3	solid	20%	n.p.	8							50			Moderate			Gautheron et al. (1994)
Promethazine hydrochloride	58-33-3	solid	20%	n.p.	9							139			Severe			Gautheron et al. (1994)
Promethazine hydrochloride	58-33-3	solid	20%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Promethazine hydrochloride	58-33-3	solid	20%	n.p.	11							94			Severe			Gautheron et al. (1994)
Promethazine hydrochloride	58-33-3	solid	20%	n.p.	12							19			Mild			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	n.p.	1							7			Mild	Mild	Cat III	Gautheron et al. (1994)
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	n.p.	2							7			Mild			Gautheron et al. (1994)
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	n.p.	3							14			Mild			Gautheron et al. (1994)
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	n.p.	4							4			Mild			Gautheron et al. (1994)
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	n.p.	5							6			Mild			Gautheron et al. (1994)
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	n.p.	6							9			Mild			Gautheron et al. (1994)
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	n.p.	7							6			Mild			Gautheron et al. (1994)
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	n.p.	8							11			Mild			Gautheron et al. (1994)
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	n.p.	9							6			Mild			Gautheron et al. (1994)
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	n.p.	11							12			Mild			Gautheron et al. (1994)
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	n.p.	12							5			Mild			Gautheron et al. (1994)
Pyridine	110-86-1	liquid	100%	n.p.	1							102			Severe	Severe	Cat I	Gautheron et al. (1994)
Pyridine	110-86-1	liquid	100%	n.p.	2							123			Severe			Gautheron et al. (1994)
Pyridine	110-86-1	liquid	100%	n.p.	3							186			Severe			Gautheron et al. (1994)
Pyridine	110-86-1	liquid	100%	n.p.	4							79			Severe			Gautheron et al. (1994)
Pyridine	110-86-1	liquid	100%	n.p.	5							102			Severe			Gautheron et al. (1994)
Pyridine	110-86-1	liquid	100%	n.p.	6							77			Severe			Gautheron et al. (1994)
Pyridine	110-86-1	liquid	100%	n.p.	7							124			Severe			Gautheron et al. (1994)
Pyridine	110-86-1	liquid	100%	n.p.	8							132			Severe			Gautheron et al. (1994)
Pyridine	110-86-1	liquid	100%	n.p.	9							105			Severe			Gautheron et al. (1994)
Pyridine	110-86-1	liquid	100%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Pyridine	110-86-1	liquid	100%	n.p.	11							96			Severe			Gautheron et al. (1994)
Pyridine	110-86-1	liquid	100%	n.p.	12							115			Severe			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Quinacrine	69-05-6	solid	20%	n.p.	1							17			Mild	Moderate	Cat III	Gautheron et al. (1994)
Quinacrine	69-05-6	solid	20%	n.p.	2							29			Moderate			Gautheron et al. (1994)
Quinacrine	69-05-6	solid	20%	n.p.	3							8			Mild			Gautheron et al. (1994)
Quinacrine	69-05-6	solid	20%	n.p.	4							46			Moderate			Gautheron et al. (1994)
Quinacrine	69-05-6	solid	20%	n.p.	5							52			Moderate			Gautheron et al. (1994)
Quinacrine	69-05-6	solid	20%	n.p.	6							24			Mild			Gautheron et al. (1994)
Quinacrine	69-05-6	solid	20%	n.p.	7							15			Moderate			Gautheron et al. (1994)
Quinacrine	69-05-6	solid	20%	n.p.	8							18			Moderate			Gautheron et al. (1994)
Quinacrine	69-05-6	solid	20%	n.p.	9							58			Severe			Gautheron et al. (1994)
Quinacrine	69-05-6	solid	20%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Quinacrine	69-05-6	solid	20%	n.p.	11							3			Not Labeled			Gautheron et al. (1994)
Quinacrine	69-05-6	solid	20%	n.p.	12							72			Severe			Gautheron et al. (1994)
Sodium oxalate	62-76-0	solid	20%	n.p.	1							2			Not Labeled	Mild	Cat III	Gautheron et al. (1994)
Sodium oxalate	62-76-0	solid	20%	n.p.	2							2			Not Labeled			Gautheron et al. (1994)
Sodium oxalate	62-76-0	solid	20%	n.p.	3							9			Mild			Gautheron et al. (1994)
Sodium oxalate	62-76-0	solid	20%	n.p.	4							5			Mild			Gautheron et al. (1994)
Sodium oxalate	62-76-0	solid	20%	n.p.	5							3			Not Labeled			Gautheron et al. (1994)
Sodium oxalate	62-76-0	solid	20%	n.p.	6							2			Not Labeled			Gautheron et al. (1994)
Sodium oxalate	62-76-0	solid	20%	n.p.	7							4			Mild			Gautheron et al. (1994)
Sodium oxalate	62-76-0	solid	20%	n.p.	8							3			Not Labeled			Gautheron et al. (1994)
Sodium oxalate	62-76-0	solid	20%	n.p.	9							3			Not Labeled			Gautheron et al. (1994)
Sodium oxalate	62-76-0	solid	20%	n.p.	10							9			Mild			Gautheron et al. (1994)
Sodium oxalate	62-76-0	solid	20%	n.p.	11							11			Mild			Gautheron et al. (1994)
Sodium oxalate	62-76-0	solid	20%	n.p.	12							4			Mild			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	n.p.	1							5			Mild	Mild	Cat III	Gautheron et al. (1994)
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	n.p.	2							1			Not Labeled			Gautheron et al. (1994)
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	n.p.	3							2			Not Labeled			Gautheron et al. (1994)
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	n.p.	4							6			Mild			Gautheron et al. (1994)
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	n.p.	5							0			Not Labeled			Gautheron et al. (1994)
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	n.p.	6							4			Mild			Gautheron et al. (1994)
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	n.p.	7							2			Not Labeled			Gautheron et al. (1994)
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	n.p.	8							19			Mild			Gautheron et al. (1994)
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	n.p.	9							3			Not Labeled			Gautheron et al. (1994)
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	n.p.	11							18			Mild			Gautheron et al. (1994)
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	n.p.	12							6			Mild			Gautheron et al. (1994)
Thiourea	62-56-6	solid	20%	n.p.	1							146			Severe	Severe	Cat I	Gautheron et al. (1994)
Thiourea	62-56-6	solid	20%	n.p.	2							175			Severe			Gautheron et al. (1994)
Thiourea	62-56-6	solid	20%	n.p.	3							169			Severe			Gautheron et al. (1994)
Thiourea	62-56-6	solid	20%	n.p.	4							152			Severe			Gautheron et al. (1994)
Thiourea	62-56-6	solid	20%	n.p.	5							140			Severe			Gautheron et al. (1994)
Thiourea	62-56-6	solid	20%	n.p.	6							120			Severe			Gautheron et al. (1994)
Thiourea	62-56-6	solid	20%	n.p.	7							129			Severe			Gautheron et al. (1994)
Thiourea	62-56-6	solid	20%	n.p.	8							173			Severe			Gautheron et al. (1994)
Thiourea	62-56-6	solid	20%	n.p.	9							151			Severe			Gautheron et al. (1994)
Thiourea	62-56-6	solid	20%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Thiourea	62-56-6	solid	20%	n.p.	11							203			Severe			Gautheron et al. (1994)
Thiourea	62-56-6	solid	20%	n.p.	12							104			Severe			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Triethanolamine	102-71-6	liquid	100%	n.p.	1							2			Not Labeled	Not Labeled	Cat IV	Gautheron et al. (1994)
Triethanolamine	102-71-6	liquid	100%	n.p.	2							4			Mild			Gautheron et al. (1994)
Triethanolamine	102-71-6	liquid	100%	n.p.	3							0			Not Labeled			Gautheron et al. (1994)
Triethanolamine	102-71-6	liquid	100%	n.p.	4							0			Not Labeled			Gautheron et al. (1994)
Triethanolamine	102-71-6	liquid	100%	n.p.	5							-1			Not Labeled			Gautheron et al. (1994)
Triethanolamine	102-71-6	liquid	100%	n.p.	6							1			Not Labeled			Gautheron et al. (1994)
Triethanolamine	102-71-6	liquid	100%	n.p.	7							1			Not Labeled			Gautheron et al. (1994)
Triethanolamine	102-71-6	liquid	100%	n.p.	8							3			Not Labeled			Gautheron et al. (1994)
Triethanolamine	102-71-6	liquid	100%	n.p.	9							3			Not Labeled			Gautheron et al. (1994)
Triethanolamine	102-71-6	liquid	100%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Triethanolamine	102-71-6	liquid	100%	n.p.	11							5			Mild			Gautheron et al. (1994)
Triethanolamine	102-71-6	liquid	100%	n.p.	12							6			Mild			Gautheron et al. (1994)
Triton X-155	9010-44-0	surfactant	10%	n.p.	1							-1			Not Labeled	Not Labeled	Cat IV	Gautheron et al. (1994)
Triton X-155	9010-44-0	surfactant	10%	n.p.	2							1			Not Labeled			Gautheron et al. (1994)
Triton X-155	9010-44-0	surfactant	10%	n.p.	3							-1			Not Labeled			Gautheron et al. (1994)
Triton X-155	9010-44-0	surfactant	10%	n.p.	4							0			Not Labeled			Gautheron et al. (1994)
Triton X-155	9010-44-0	surfactant	10%	n.p.	5							2			Not Labeled			Gautheron et al. (1994)
Triton X-155	9010-44-0	surfactant	10%	n.p.	6							2			Not Labeled			Gautheron et al. (1994)
Triton X-155	9010-44-0	surfactant	10%	n.p.	7							0			Not Labeled			Gautheron et al. (1994)
Triton X-155	9010-44-0	surfactant	10%	n.p.	8							2			Not Labeled			Gautheron et al. (1994)
Triton X-155	9010-44-0	surfactant	10%	n.p.	9							3			Not Labeled			Gautheron et al. (1994)
Triton X-155	9010-44-0	surfactant	10%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Triton X-155	9010-44-0	surfactant	10%	n.p.	11							-2			Not Labeled			Gautheron et al. (1994)
Triton X-155	9010-44-0	surfactant	10%	n.p.	12							0			Not Labeled			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
4-Carboxybenzaldehyde	619-66-9	solid	20%	95	1 (1)	53.7	3	4.6	0.012	3	0.012	53.9	3	4.9	Moderate	Moderate	Cat II	Southee (1998)
4-Carboxybenzaldehyde	619-66-9	solid	20%	95	1 (2)	47.7	3	3.5	0.002	3	0.02	47.7	3	3.4	Moderate			Southee (1998)
4-Carboxybenzaldehyde	619-66-9	solid	20%	95	2 (1)	46.3	3	3.2	0.05	3	0.021	47.1	3	3.1	Moderate			Southee (1998)
4-Carboxybenzaldehyde	619-66-9	solid	20%	95	2 (2)	46.4	3	2.9	0.058	3	0.014	47.2	3	2.9	Moderate			Southee (1998)
4-Carboxybenzaldehyde	619-66-9	solid	20%	95	3 (1)	42	3	4.5	0.013	3	0.016	42.2	3	4.3	Moderate			Southee (1998)
4-Carboxybenzaldehyde	619-66-9	solid	20%	95	3 (2)	41.3	3	4.0	0.035	3	0.006	41.8	3	3.9	Moderate			Southee (1998)
Ammonium nitrate	6484-52-2	n.p.	100%	n.p.	1 (1)	4.3	3	2.1	0.037	3	0.036	4.9	3	2.4	Mild	Mild	Cat III	Southee (1998)
Ammonium nitrate	6484-52-2	n.p.	100%	n.p.	1 (2)	5.0	3	1.2	0.059	3	0.031	5.9	3	1.4	Mild			Southee (1998)
Ammonium nitrate	6484-52-2	n.p.	100%	n.p.	2 (1)	1.6	3	1.2	0.153	3	0.059	3.9	3	1.8	Mild			Southee (1998)
Ammonium nitrate	6484-52-2	n.p.	100%	n.p.	2 (2)	2.0	3	0.6	0.107	3	0.044	3.6	3	1	Mild			Southee (1998)
Ammonium nitrate	6484-52-2	n.p.	100%	n.p.	3 (1)	3.7	3	0.6	0.100	3	0.033	5.2	3	0.6	Mild			Southee (1998)
Ammonium nitrate	6484-52-2	n.p.	100%	n.p.	3 (2)	4.3	3	0.6	0.158	3	0.07	6.7	3	1.5	Mild			Southee (1998)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	1 (1)	84.0	3	3.8	7.408	3	0.903	195.2	3	11.3	Very severe	Very Severe	Cat I	Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	1 (2)	85.6	3	3.2	3.305	3	0.225	135.2	3	5.2	Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	1 (3)	82.0	3	1.7	3.729	3	0.25	137.9	3	2.3	Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	1 (4)	85.0	3	5.2	4.766	3	1.132	156.5	3	18.6	Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	1 (5)	87.7	3	1.7	3.354	3	0.108	138.0	3	0.1	Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	1 (6)	91.7	3	7.0	5.67	3	1.096	176.8			Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	1 (7)	98.3	3	2.6	5.645	3	0.523	183.0			Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	1 (8)	87.7	3	2.9	5.848	3	0.581	175.4			Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	2 (1)	88.0	3	7.5	4.426	3	0.623	154.4	3	11.1	Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	2 (2)	94.6	3	10.4	4.148	3	0.662	156.9	3	18.6	Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	2 (3)	87.0	3	7.5	4.252	3	0.069	150.8	3	7.1	Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	2 (4)	93.0	3	3.0	4.278	3	1.058	157.2	3	18.0	Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	2 (5)	98.3	3	2.3	3.972	3	0.360	157.9	3	3.4	Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	2 (6)	95.7	3	5.0	4.129	3	0.581	157.0			Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	2 (7)	98.0	3	5.1	4.144	3	0.232	160.2			Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	3 (1)	96.7	3	2.0	4.015	3	1.011	156.9	3	17.1	Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	3 (2)	92.6	3	11.8	4.719	3	1.547	163.4	3	16.2	Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	3 (3)	105.0	3	6.1	4.316	3	0.320	169.7	3	10.2	Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	3 (4)	95.3	3	4.0	4.497	3	1.007	162.8	3	11.4	Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	3 (5)	92.3	3	7.2	3.948	3	0.231	151.6	3	7.7	Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	3 (6)	93.7	3	4.9	4.624	3	1.708	163.1	3	22.1	Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	3 (7)	100.7	3	2.5	4.473	3	0.619	167.8	3	7.8	Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	3 (8)	96.7	3	2.0	9.016	3	1.011	156.9	3	17.1	Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	3 (9)	97.3	3	5.1	4.183	3	0.514	160.0	3	8.2	Very severe			Southee (1998)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Ethanol	64-17-5	liquid	100%	n.p.	1 (1)	17.6	3	2.3	1.265	3	0.252	36.6	3	6.0	Moderate	Moderate	Cat II	Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	1 (2)	16.4	3	5.5	1.415	3	0.389	37.6	3	10.8	Moderate			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	1 (3)	13.7	3	1.5	1.062	3	0.322	29.6	3	6.4	Moderate			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	1 (4)	12.7	3	1.0	1.933	3	0.397	41.7	3	5.8	Moderate			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	1 (5)	14.7	3	2.1	1.125	3	0.162	31.5	3	4.5	Moderate			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	1 (6)	12.7	3	14.9	1.995	3	0.035	42.6			Moderate			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	1 (7)	18.7	3	1.5	2.445	3	0.733	55.4			Severe			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	2 (1)	13.3	3	1.0	2.626	3	0.909	52.7	3	12.8	Moderate			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	2 (2)	17.0	3	2.3	2.504	3	0.703	54.5	3	8.3	Moderate			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	2 (3)	16.3	3	4.9	3.025	3	0.699	61.7	3	7.8	Severe			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	2 (4)	17.3	3	1.5	2.857	3	0.250	60.2	3	4.9	Severe			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	2 (5)	14.7	3	2.1	2.636	3	0.427	54.2	3	5.0	Moderate			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	2 (6)	17.6	3	0.6	3.718	3	0.798	73.4			Severe			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	2 (7)	15.0	3	2.6	3.267	3	0.545	64.0			Severe			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	2 (8)	13.0	3	0.6	2.561	3	0.867	51.4			Moderate			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	3 (1)	16.6	3	2.1	2.027	3	1.026	47.0	3	14.3	Moderate			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	3 (2)	18.0	3	2.9	1.831	3	0.061	45.4	3	2.0	Moderate			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	3 (3)	19.3	3	2.6	1.673	3	0.071	44.4	3	3.0	Moderate			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	3 (4)	22.0	3	2.6	1.583	3	0.426	45.7	3	8.5	Moderate			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	3 (5)	18.6	3	1.5	2.395	3	0.380	54.6	3	4.5	Moderate			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	3 (6)	17.0	3	1.2	1.853	3	0.268	44.8	3	5.1	Moderate			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	3 (7)	19.3	3	3.8	1.527	3	0.344	42.2	3	8.8	Moderate			Southee (1998)
Butyl cellusolve	111-76-2	liquid	100%	n.p.	1 (1)	39	3	7.8	4.625	3	0.471	108.3	3	12.9	Very severe	Very Severe	Cat I	Southee (1998)
Butyl cellusolve	111-76-2	liquid	100%	n.p.	1 (2)	43	3	4.0	4.589	3	0.418	111.8	3	5.5	Very severe			Southee (1998)
Butyl cellusolve	111-76-2	liquid	100%	n.p.	2 (1)	29.6	3	1.5	4.213	3	0.78	92.8	3	13	Very severe			Southee (1998)
Butyl cellusolve	111-76-2	liquid	100%	n.p.	2 (2)	31.3	3	2.3	4.526	3	0.864	99.2	3	10.7	Very severe			Southee (1998)
Butyl cellusolve	111-76-2	liquid	100%	n.p.	3 (1)	37.7	3	1.0	3.813	3	0.933	94.9	3	13.8	Very severe			Southee (1998)
Butyl cellusolve	111-76-2	liquid	100%	n.p.	3 (2)	37.7	3	6.1	4.031	3	1.206	98.2	3	21.6	Very severe			Southee (1998)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Glycerol	56-81-5	liquid	100%	>99.5	1 (1)	0.6	3	0.6	-0.005	3	0.002	0.6	3	0.6	Not Labeled	Not Labeled	Not Labeled	Southee (1998)
Glycerol	56-81-5	liquid	100%	>99.5	1 (2)	0.3	3	1.0	-0.003	3	0.002	0.3	3	1.0	Not Labeled			Southee (1998)
Glycerol	56-81-5	liquid	100%	>99.5	2 (1)	0.6	3	0.6	0.012	3	0.007	0.8	3	0.6	Not Labeled			Southee (1998)
Glycerol	56-81-5	liquid	100%	>99.5	2 (2)	0.7	3	0.6	0.008	3	0.009	0.8	3	0.7	Not Labeled			Southee (1998)
Glycerol	56-81-5	liquid	100%	>99.5	3 (1)	1.0	3	0.6	-0.003	3	0.005	1.0	3	0.6	Not Labeled			Southee (1998)
Glycerol	56-81-5	liquid	100%	>99.5	3 (2)	0.7	3	0.0	0.007	3	0.011	0.8	3	0.2	Not Labeled			Southee (1998)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	1 (1)	13.3	3	2.0	0.654	3	0.273	23.1	3	5.9	Mild	Moderate	Cat II	Southee (1998)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	1 (2)	9.7	3	4.2	0.499	3	0.109	17.2	3	5.8	Mild			Southee (1998)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	2 (1)	13.7	3	3.2	1.398	3	0.601	34.6	3	12.1	Moderate			Southee (1998)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	2 (2)	13.0	3	4.4	1.743	3	0.871	39.1	3	16.4	Moderate			Southee (1998)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	3 (1)	17.3	3	1.0	0.958	3	0.100	31.7	3	2.3	Moderate			Southee (1998)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	3 (2)	17.7	3	2.1	0.818	3	0.607	29.9	3	11.2	Moderate			Southee (1998)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Imidazole	288-32-4	solid	20%	n.p.	1 (1)	91.3	3	2.1	3.379	3	0.106	142.0	3	3.0	Very severe	Very Severe	Very Severe	Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	1 (2)	88.0	3	7.5	3.306	3	0.597	137.6	3	6.8	Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	1 (3)	73.7	3	10.1	2.565	3	1.063	112.2	3	24.7	Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	1 (4)	86.0	3	9.6	3.006	3	1.078	131.1	3	6.7	Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	1 (5)	97.0	3	15.5	3.241	3	0.233	145.6	3	12.0	Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	1 (6)	115.3	3	9.1	3.150	3	0.181	162.6	3		Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	1 (7)	70.3	3	4.5	3.681	3	0.691	125.5	3		Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	2 (1)	85.7	3	9.8	3.490	3	0.309	138.1	3	13.0	Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	2 (2)	88.0	3	13.0	3.471	3	0.381	140.1	3	11.9	Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	2 (3)	86.3	3	6.0	3.240	3	0.651	134.9	3	9.4	Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	2 (4)	92.3	3	7.9	4.324	3	1.048	157.2	3	12.5	Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	2 (5)	88.0	3	16.7	3.308	3	0.695	137.6	3	6.8	Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	2 (6)	97.3	3	12.9	3.709	3	0.866	152.9			Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	2 (7)	100.0	3	9.1	3.316	3	0.183	148.7			Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	3 (1)	83.0	3	14.8	3.774	3	0.828	139.6	3	26.0	Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	3 (2)	91.7	3	9.3	3.232	3	0.702	140.1	3	18.9	Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	3 (3)	80.4	3	3.1	2.907	3	0.642	124.0	3	6.9	Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	3 (4)	82.3	3	2.1	3.093	3	0.635	128.7	3	8.2	Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	3 (5)	76.6	3	8.3	3.118	3	0.464	123.4	3	14.8	Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	3 (6)	76.3	3	8.7	2.862	3	0.292	121.2	3	4.6	Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	3 (7)	77.3	3	2.0	3.602	3	0.413	131.3	3	8.2	Very severe			Southee (1998)
Methyl ethyl ketone	78-93-3	liquid	100%	99	1 (1)	47.6	3	5.9	1.706	3	0.679	73.3	3	15.9	Severe	Severe	Cat II	Southee (1998)
Methyl ethyl ketone	78-93-3	liquid	100%	99	1 (2)	48	3	2.1	1.32	3	0.303	67.8	3	5.7	Severe			Southee (1998)
Methyl ethyl ketone	78-93-3	liquid	100%	99	2 (1)	61	3	2.9	3.183	3	0.86	108.7	3	11.9	Very severe			Southee (1998)
Methyl ethyl ketone	78-93-3	liquid	100%	99	2 (2)	62	3	6.7	2.648	3	1.074	101.7	3	21.1	Very severe			Southee (1998)
Methyl ethyl ketone	78-93-3	liquid	100%	99	3 (1)	55.7	3	5.0	0.972	3	0.479	70.2	3	3.5	Severe			Southee (1998)
Methyl ethyl ketone	78-93-3	liquid	100%	99	3 (2)	54.4	3	1.5	1.278	3	0.359	73.5	3	6.4	Severe			Southee (1998)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Parafluoraniline	371-40-4	liquid	100%	99	1 (1)	15.3	3	1.0	1.044	3	0.413	31	3	7.2	Moderate	Moderate	Cat II	Southee (1998)
Parafluoraniline	371-40-4	liquid	100%	99	1 (2)	16.3	3	3.5	1.243	3	0.287	35	3	6.2	Moderate			Southee (1998)
Parafluoraniline	371-40-4	liquid	100%	99	2 (1)	13.3	3	2.1	1.663	3	0.372	38.3	3	7.5	Moderate			Southee (1998)
Parafluoraniline	371-40-4	liquid	100%	99	2 (2)	16.0	3	4.6	1.432	3	0.531	37.5	3	12.2	Moderate			Southee (1998)
Parafluoraniline	371-40-4	liquid	100%	99	3 (1)	11.0	3	1.0	0.738	3	0.154	22.1	3	2.7	Mild			Southee (1998)
Parafluoraniline	371-40-4	liquid	100%	99	3 (2)	15.4	3	1.2	0.7	3	0.151	28.9	3	3.4	Moderate			Southee (1998)
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	100	1 (1)	10.7	3	2.6	0.034	3	0.044	11.2	3	3.2	Mild	Mild	Cat III	Southee (1998)
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	100	1 (2)	7.0	3	0.6	0.023	3	0.026	7.4	3	0.6	Mild			Southee (1998)
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	100	2 (1)	5.0	3	1.7	0.013	3	0.012	5.2	3	1.9	Mild			Southee (1998)
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	100	2 (2)	3.4	3	1.5	0.016	3	0.015	3.6	3	1.6	Mild			Southee (1998)
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	100	3 (1)	7.3	3	4.4	0.028	3	0.014	7.7	3	4.2	Mild			Southee (1998)
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	100	3 (2)	5.6	3	0.6	0.04	3	0.051	6.2	3	0.7	Mild			Southee (1998)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Sodium hydroxide (10%)	1310-73-2	liquid	10%	n.p.	1 (1)	176.7	3	31.4	4.551	3	1.019	245.0	3	28.7	Very severe	Very Severe	Cat I	Southee (1998)
Sodium hydroxide (10%)	1310-73-2	liquid	10%	n.p.	1 (2)	172.0	3	1.7	3.676	3	0.201	227.1	3	3.4	Very severe			Southee (1998)
Sodium hydroxide (10%)	1310-73-2	liquid	10%	n.p.	2 (1)	170.0	3	20.7	4.755	3	0.586	241.3	3	11.9	Very severe			Southee (1998)
Sodium hydroxide (10%)	1310-73-2	liquid	10%	n.p.	2 (2)	166.7	3	12.6	4.590	3	0.405	235.5	3	7.3	Very severe			Southee (1998)
Sodium hydroxide (10%)	1310-73-2	liquid	10%	n.p.	3 (1)	124.0	3	13.7	4.604	3	0.380	193.1	3	19.0	Very severe			Southee (1998)
Sodium hydroxide (10%)	1310-73-2	liquid	10%	n.p.	3 (2)	165.3	3	21.2	3.303	3	0.388	214.9	3	15.5	Very severe			Southee (1998)
Sodium lauryl sulfate (15%)	151-21-3	liquid	15%	98	1 (1)	-0.8	3	0.0	0.408	3	0.024	5.4	3	0.4	Mild	Mild	Cat III	Southee (1998)
Sodium lauryl sulfate (15%)	151-21-3	liquid	15%	98	1 (2)	0.0	3	0.6	0.348	3	0.182	5.2	3	2.7	Mild			Southee (1998)
Sodium lauryl sulfate (15%)	151-21-3	liquid	15%	98	2 (1)	0.7	3	1.0	1.012	3	0.461	15.9	3	7.6	Mild			Southee (1998)
Sodium lauryl sulfate (15%)	151-21-3	liquid	15%	98	2 (2)	1.0	3	0.6	1.086	3	0.083	17.3	3	1.7	Mild			Southee (1998)
Sodium lauryl sulfate (15%)	151-21-3	liquid	15%	98	3 (1)	0.7	3	0.6	0.518	3	0.11	8.7	3	1.4	Mild			Southee (1998)
Sodium lauryl sulfate (15%)	151-21-3	liquid	15%	98	3 (2)	1.3	3	0.6	0.283	3	0.064	5.6	3	1.5	Mild			Southee (1998)
Sodium oxalate	62-76-0	solid	20%	99	1 (1)	8.4	3	1.2	0.128	3	0.16	10.3	3	1.4	Mild	Not Labeled	Cat III	Southee (1998)
Sodium oxalate	62-76-0	solid	20%	99	1 (2)	3.4	3	0.6	0.071	3	0.03	4.4	3	1.0	Mild			Southee (1998)
Sodium oxalate	62-76-0	solid	20%	99	2 (1)	-1.0	3	1.7	0.05	3	0.054	-0.3	3	1.5	Not Labeled			Southee (1998)
Sodium oxalate	62-76-0	solid	20%	99	2 (2)	-1.0	3	2.1	0.055	3	0.012	-0.1	3	2.1	Not Labeled			Southee (1998)
Sodium oxalate	62-76-0	solid	20%	99	3 (1)	2.0	3	0.6	0.051	3	0.032	2.7	3	0.9	Not Labeled			Southee (1998)
Sodium oxalate	62-76-0	solid	20%	99	3 (2)	2.3	3	1.0	0.15	3	0.022	4.5	3	1.3	Mild			Southee (1998)
Triton X-100 (5%)	9002-93-1	liquid	5%	98	1 (1)	3.3	3	1.0	0.023	3	0.004	3.7	3	1.1	Mild	Mild	Cat III	Southee (1998)
Triton X-100 (5%)	9002-93-1	liquid	5%	98	1 (2)	1.3	3	1.0	0.035	3	0.006	1.8	3	1.0	Not Labeled			Southee (1998)
Triton X-100 (5%)	9002-93-1	liquid	5%	98	2 (1)	1.4	3	0.6	0.298	3	0.123	5.8	3	2.4	Mild			Southee (1998)
Triton X-100 (5%)	9002-93-1	liquid	5%	98	2 (2)	0.0	3	0.6	0.226	3	0.086	3.4	3	1.0	Mild			Southee (1998)
Triton X-100 (5%)	9002-93-1	liquid	5%	98	3 (1)	2.7	3	1.0	0.023	3	0.009	3.0	3	1.1	Not Labeled			Southee (1998)
Triton X-100 (5%)	9002-93-1	liquid	5%	98	3 (2)	1.4	3	0.6	0.038	3	0.013	1.9	3	0.6	Not Labeled			Southee (1998)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Tween 20	9005-64-5	liquid	100%	98	1 (1)	0.3	3	0.0	0.003	3	0.012	0.3	3	0.2	Not Labeled	Not Labeled	Cat IV	Southee (1998)
Tween 20	9005-64-5	liquid	100%	98	1 (2)	0.0	3	1.5	0.004	3	0.01	0.0	3	1.6	Not Labeled			Southee (1998)
Tween 20	9005-64-5	liquid	100%	98	2 (1)	0.4	3	0.6	0.001	3	0.002	0.4	3	0.6	Not Labeled			Southee (1998)
Tween 20	9005-64-5	liquid	100%	98	2 (2)	0.4	3	0.6	0.003	3	0.008	0.4	3	0.5	Not Labeled			Southee (1998)
Tween 20	9005-64-5	liquid	100%	98	3 (1)	0.0	3	0.0	0.022	3	0.018	0.3	3	0.3	Not Labeled			Southee (1998)
Tween 20	9005-64-5	liquid	100%	98	3 (2)	0.0	3	1.0	0.001	3	0.022	0.0	3	1.3	Not Labeled			Southee (1998)
1-1 (#1)	-	liquid	100%	n.p.	-							83.6			Severe	Severe	Cat I	Swanson and Harbell (2000)
1-2 (#2)	-	liquid	100%	n.p.	-							12.4			Mild	Mild	Cat III	Swanson and Harbell (2000)
1-3 (#3)	-	liquid	100%	n.p.	-							29.6			Moderate	Moderate	Cat II	Swanson and Harbell (2000)
2-4 (#4)	-	liquid	100%	n.p.	-							7.3			Mild	Mild	Cat III	Swanson and Harbell (2000)
2-7 (#7)	-	liquid	100%	n.p.	-							21.4			Moderate	Moderate	Cat III	Swanson and Harbell (2000)
2-8 (#8)	-	liquid	100%	n.p.	-							31.8			Moderate	Moderate	Cat II	Swanson and Harbell (2000)
Benchmark-Group 1 (#12)	-	liquid	100%	n.p.	-							60.1			Severe	Severe	Cat II	Swanson and Harbell (2000)
Benchmark-Group 2 (#13)	-	liquid	100%	n.p.	-							60.1			Severe	Severe	Cat II	Swanson and Harbell (2000)
All Purpose Cleaner (#5)	-	liquid	100%	n.p.	-	102.5	5		1.252	5		121.3	5		Severe	Severe	Cat I	Swanson et al. (1995)
All Purpose Cleaner (#7)	-	liquid	100%	n.p.	-	348.1	5		3.013	5		393.3	5		Severe	Severe	Cat I	Swanson et al. (1995)
Bathroom Cleaner (#6)	-	liquid	100%	n.p.	-	64	5		0.95	5		78.3	5		Severe	Severe	Cat I	Swanson et al. (1995)
Cleaner/Degreaser (#13)	-	liquid	100%	n.p.	-	314.3	5		2.623	5		353.6	5		Severe	Severe	Cat I	Swanson et al. (1995)
Degreaser (#16)	-	liquid	100%	n.p.	-	225.4	5		2.022	5		255.7	5		Severe	Severe	Cat I	Swanson et al. (1995)
Floor Cleaner (#10)	-	liquid	100%	n.p.	-	45.2	5		1.675	5		70.3	5		Severe	Severe	Cat II	Swanson et al. (1995)
Floor Cleaner (#2)	-	liquid	100%	n.p.	-	-2.1	5		0.119	5		-0.3	5		Not Labeled	Not Labeled	Cat IV	Swanson et al. (1995)
Floor Stripper (#14)	-	liquid	100%	n.p.	-	122.5	5		2.318	5		157.3	5		Severe	Severe	Cat I	Swanson et al. (1995)
Floor Stripper (#17)	-	liquid	100%	n.p.	-	180.5	5		2.38	5		216.2	5		Severe	Severe	Cat I	Swanson et al. (1995)
Floor Stripper (#18)	-	liquid	100%	n.p.	-	407.1	5		2.481	5		444.3	5		Severe	Severe	Cat I	Swanson et al. (1995)
General Cleaner (#11)	-	liquid	100%	n.p.	-	77.9	5		0.359	5		83.3	5		Severe	Severe	Cat I	Swanson et al. (1995)
General Cleaner (#12)	-	liquid	100%	n.p.	-	95.5	5		1.197	5		113.5	5		Severe	Severe	Cat I	Swanson et al. (1995)
Glass Cleaner (#19)	-	liquid	100%	n.p.	-	98.3	5		2.499	5		135.8	5		Severe	Severe	Cat I	Swanson et al. (1995)
Heavy Duty Cleaner (#15)	-	liquid	100%	n.p.	-	323.3	5		2.24	5		357.1	5		Severe	Severe	Cat I	Swanson et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Heavy Duty Cleaner/Degreaser (#9)	-	liquid	100%	n.p.	-	315.4	5		2.619	5		354.7	5		Severe	Severe	Cat I	Swanson et al. (1995)
Meat Room Degreaser (#3)	-	liquid	100%	n.p.	-	99.3	5		2.733	5		140.3	5		Severe	Severe	Cat I	Swanson et al. (1995)
Metal Cleaner (#20)	-	liquid	100%	n.p.	-	344.2	5		3.182	5		391.9	5		Severe	Severe	Cat I	Swanson et al. (1995)
Pot and Pan Cleaner (#8)	-	liquid	100%	n.p.	-	-1.8	5		0.078	5		-0.6	5		Not Labeled	Not Labeled	Cat IV	Swanson et al. (1995)
Toilet Bowl Cleaner (#1)	-	liquid	100%	n.p.	-	8.700	5		0.323	5		13.5	5		Mild	Mild	Cat III	Swanson et al. (1995)
Toilet Bowl Cleaner (#4)	-	liquid	100%	n.p.	-	10.5	5		0.303	5		15	5		Mild	Mild	Cat III	Swanson et al. (1995)

Abbreviations: CASRN=Chemical Abstract Services Registry Number; SCNM=Study Criteria Not Met; n.p.=Not Provided; n.a.=Not Applicable

[1] *In Vitro* Classification represents the BCOP ocular irritancy classification assigned for each chemical in the study for each test for a specific substance

[2] Consensus classification represents the overall BCOP ocular irritancy classification assigned for each chemical in the study based on the majority of ocular irritancy classification calls

Annex II-2

BCOP Data Sorted by Substance Name

This page intentionally left blank

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
1-1 (#1)	-	liquid	100%	n.p.	-							83.6			Severe	Severe	Cat I	Swanson and Harbell (2000)
1-2 (#2)	-	liquid	100%	n.p.	-							12.4			Mild	Mild	Cat III	Swanson and Harbell (2000)
1-3 (#3)	-	liquid	100%	n.p.	-							29.6			Moderate	Moderate	Cat II	Swanson and Harbell (2000)
1-Naphthalene acetic acid	86-87-3	solid	20%	96	1	119.4			0.095			120.8			Very severe	Very Severe	Cat II	Balls et al. (1995)
1-Naphthalene acetic acid	86-87-3	solid	20%	96	2	65.7			0.045			66.3			Severe			Balls et al. (1995)
1-Naphthalene acetic acid	86-87-3	solid	20%	96	3	41			0.065			42			Moderate			Balls et al. (1995)
1-Naphthalene acetic acid	86-87-3	solid	20%	96	4	86.67			0.137			88.73			Very severe			Balls et al. (1995)
1-Naphthalene acetic acid	86-87-3	solid	20%	96	5	70			0.168			72.5			Severe			Balls et al. (1995)
1-Naphthalene acetic acid, Na salt	61-31-4	solid	20%	95	1	73.3			4.177			136			Very severe	Very Severe	Cat I	Balls et al. (1995)
1-Naphthalene acetic acid, Na salt	61-31-4	solid	20%	95	2	83			4.124			144.9			Very severe			Balls et al. (1995)
1-Naphthalene acetic acid, Na salt	61-31-4	solid	20%	95	3	73			5.864			161			Very severe			Balls et al. (1995)
1-Naphthalene acetic acid, Na salt	61-31-4	solid	20%	95	4	108			3.55			161.2			Very severe			Balls et al. (1995)
1-Naphthalene acetic acid, Na salt	61-31-4	solid	20%	95	5	94.7			3.222			143			Very severe			Balls et al. (1995)
1-Nitropropane	108-03-2	liquid	100%	n.p.	1							11			Mild	Mild	Cat III	Gautheron et al. (1994)
1-Nitropropane	108-03-2	liquid	100%	n.p.	2							8			Mild			Gautheron et al. (1994)
1-Nitropropane	108-03-2	liquid	100%	n.p.	3							9			Mild			Gautheron et al. (1994)
1-Nitropropane	108-03-2	liquid	100%	n.p.	4							4			Mild			Gautheron et al. (1994)
1-Nitropropane	108-03-2	liquid	100%	n.p.	5							6			Mild			Gautheron et al. (1994)
1-Nitropropane	108-03-2	liquid	100%	n.p.	6							7			Mild			Gautheron et al. (1994)
1-Nitropropane	108-03-2	liquid	100%	n.p.	7							6			Mild			Gautheron et al. (1994)
1-Nitropropane	108-03-2	liquid	100%	n.p.	8							6			Mild			Gautheron et al. (1994)
1-Nitropropane	108-03-2	liquid	100%	n.p.	9							17			Mild			Gautheron et al. (1994)
1-Nitropropane	108-03-2	liquid	100%	n.p.	10							4			Mild			Gautheron et al. (1994)
1-Nitropropane	108-03-2	liquid	100%	n.p.	11							6			Mild			Gautheron et al. (1994)
1-Nitropropane	108-03-2	liquid	100%	n.p.	12							7			Mild			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
1-Phenyl-3-pyrazolidone	92-43-3	solid	20%	n.p.	1							7			Mild	Mild	Cat III	Gautheron et al. (1994)
1-Phenyl-3-pyrazolidone	92-43-3	solid	20%	n.p.	2							12			Mild			Gautheron et al. (1994)
1-Phenyl-3-pyrazolidone	92-43-3	solid	20%	n.p.	3							15			Mild			Gautheron et al. (1994)
1-Phenyl-3-pyrazolidone	92-43-3	solid	20%	n.p.	4							9			Mild			Gautheron et al. (1994)
1-Phenyl-3-pyrazolidone	92-43-3	solid	20%	n.p.	5							28			Moderate			Gautheron et al. (1994)
1-Phenyl-3-pyrazolidone	92-43-3	solid	20%	n.p.	6							6			Mild			Gautheron et al. (1994)
1-Phenyl-3-pyrazolidone	92-43-3	solid	20%	n.p.	7							6			Mild			Gautheron et al. (1994)
1-Phenyl-3-pyrazolidone	92-43-3	solid	20%	n.p.	8							16			Mild			Gautheron et al. (1994)
1-Phenyl-3-pyrazolidone	92-43-3	solid	20%	n.p.	9							13			Mild			Gautheron et al. (1994)
1-Phenyl-3-pyrazolidone	92-43-3	solid	20%	n.p.	10							15			Mild			Gautheron et al. (1994)
1-Phenyl-3-pyrazolidone	92-43-3	solid	20%	n.p.	11							13			Mild			Gautheron et al. (1994)
1-Phenyl-3-pyrazolidone	92-43-3	solid	20%	n.p.	12							15			Mild			Gautheron et al. (1994)
1,2,3-Trichloropropane	96-18-4	liquid	100%	n.p.	1							47			Moderate	Moderate	Cat II	Gautheron et al. (1994)
1,2,3-Trichloropropane	96-18-4	liquid	100%	n.p.	2							42			Moderate			Gautheron et al. (1994)
1,2,3-Trichloropropane	96-18-4	liquid	100%	n.p.	3							78			Severe			Gautheron et al. (1994)
1,2,3-Trichloropropane	96-18-4	liquid	100%	n.p.	4							28			Moderate			Gautheron et al. (1994)
1,2,3-Trichloropropane	96-18-4	liquid	100%	n.p.	5							42			Moderate			Gautheron et al. (1994)
1,2,3-Trichloropropane	96-18-4	liquid	100%	n.p.	6							47			Moderate			Gautheron et al. (1994)
1,2,3-Trichloropropane	96-18-4	liquid	100%	n.p.	7							48			Moderate			Gautheron et al. (1994)
1,2,3-Trichloropropane	96-18-4	liquid	100%	n.p.	8							24			Mild			Gautheron et al. (1994)
1,2,3-Trichloropropane	96-18-4	liquid	100%	n.p.	9							91			Severe			Gautheron et al. (1994)
1,2,3-Trichloropropane	96-18-4	liquid	100%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
1,2,3-Trichloropropane	96-18-4	liquid	100%	n.p.	11							28			Moderate			Gautheron et al. (1994)
1,2,3-Trichloropropane	96-18-4	liquid	100%	n.p.	12							47			Moderate			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
1,2,4-Trimethylbenzene	95-63-6	liquid	100%	n.p.	1							25			Mild	Mild	Cat III	Gautheron et al. (1994)
1,2,4-Trimethylbenzene	95-63-6	liquid	100%	n.p.	2							14			Mild			Gautheron et al. (1994)
1,2,4-Trimethylbenzene	95-63-6	liquid	100%	n.p.	3							26			Moderate			Gautheron et al. (1994)
1,2,4-Trimethylbenzene	95-63-6	liquid	100%	n.p.	4							11			Mild			Gautheron et al. (1994)
1,2,4-Trimethylbenzene	95-63-6	liquid	100%	n.p.	5							27			Moderate			Gautheron et al. (1994)
1,2,4-Trimethylbenzene	95-63-6	liquid	100%	n.p.	6							7			Mild			Gautheron et al. (1994)
1,2,4-Trimethylbenzene	95-63-6	liquid	100%	n.p.	7							9			Mild			Gautheron et al. (1994)
1,2,4-Trimethylbenzene	95-63-6	liquid	100%	n.p.	8							15			Mild			Gautheron et al. (1994)
1,2,4-Trimethylbenzene	95-63-6	liquid	100%	n.p.	9							21			Mild			Gautheron et al. (1994)
1,2,4-Trimethylbenzene	95-63-6	liquid	100%	n.p.	10							10			Mild			Gautheron et al. (1994)
1,2,4-Trimethylbenzene	95-63-6	liquid	100%	n.p.	11							7			Mild			Gautheron et al. (1994)
1,2,4-Trimethylbenzene	95-63-6	liquid	100%	n.p.	12							21			Mild			Gautheron et al. (1994)
2-4 (#4)	-	liquid	100%	n.p.	-							7.3			Mild	Mild	Cat III	Swanson and Harbell (2000)
2-7 (#7)	-	liquid	100%	n.p.	-							21.4			Mild	Mild	Cat III	Swanson and Harbell (2000)
2-8 (#8)	-	liquid	100%	n.p.	-							31.8			Moderate	Moderate	Cat II	Swanson and Harbell (2000)
2-Aminophenol	95-55-6	solid	20%	n.p.	1							7			Mild	Mild	Cat III	Gautheron et al. (1994)
2-Aminophenol	95-55-6	solid	20%	n.p.	2							5			Mild			Gautheron et al. (1994)
2-Aminophenol	95-55-6	solid	20%	n.p.	3							3			Not Labeled			Gautheron et al. (1994)
2-Aminophenol	95-55-6	solid	20%	n.p.	4							5			Mild			Gautheron et al. (1994)
2-Aminophenol	95-55-6	solid	20%	n.p.	5							6			Mild			Gautheron et al. (1994)
2-Aminophenol	95-55-6	solid	20%	n.p.	6							7			Mild			Gautheron et al. (1994)
2-Aminophenol	95-55-6	solid	20%	n.p.	7							5			Mild			Gautheron et al. (1994)
2-Aminophenol	95-55-6	solid	20%	n.p.	8							6			Mild			Gautheron et al. (1994)
2-Aminophenol	95-55-6	solid	20%	n.p.	9							13			Mild			Gautheron et al. (1994)
2-Aminophenol	95-55-6	solid	20%	n.p.	10							11			Mild			Gautheron et al. (1994)
2-Aminophenol	95-55-6	solid	20%	n.p.	11							5			Mild			Gautheron et al. (1994)
2-Aminophenol	95-55-6	solid	20%	n.p.	12							11			Mild			Gautheron et al. (1994)
2-Chloro-2,4,4-trimethylpentane	-	liquid	100%	n.p.	-	4.0			0.004			4.1			Mild	Mild	Cat III	Bailey et al. (2004)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
2-Ethoxyethanol	110-80-5	liquid	100%	n.p.	1							99			Severe	Severe	Cat I	Gautheron et al. (1994)
2-Ethoxyethanol	110-80-5	liquid	100%	n.p.	2							100			Severe			Gautheron et al. (1994)
2-Ethoxyethanol	110-80-5	liquid	100%	n.p.	3							128			Severe			Gautheron et al. (1994)
2-Ethoxyethanol	110-80-5	liquid	100%	n.p.	4							75			Severe			Gautheron et al. (1994)
2-Ethoxyethanol	110-80-5	liquid	100%	n.p.	5							75			Severe			Gautheron et al. (1994)
2-Ethoxyethanol	110-80-5	liquid	100%	n.p.	6							85			Severe			Gautheron et al. (1994)
2-Ethoxyethanol	110-80-5	liquid	100%	n.p.	7							94			Severe			Gautheron et al. (1994)
2-Ethoxyethanol	110-80-5	liquid	100%	n.p.	8							93			Severe			Gautheron et al. (1994)
2-Ethoxyethanol	110-80-5	liquid	100%	n.p.	9							84			Severe			Gautheron et al. (1994)
2-Ethoxyethanol	110-80-5	liquid	100%	n.p.	10							75			Severe			Gautheron et al. (1994)
2-Ethoxyethanol	110-80-5	liquid	100%	n.p.	11							101			Severe			Gautheron et al. (1994)
2-Ethoxyethanol	110-80-5	liquid	100%	n.p.	12							86			Severe			Gautheron et al. (1994)
2-Ethyl-1-hexanol	104-76-7	liquid	100%	99	2	9			1.279			28.2			Moderate	Moderate	Cat II	Balls et al. (1995)
2-Ethyl-1-hexanol	104-76-7	liquid	100%	99	3	4.3			1.761			30.7			Moderate			Balls et al. (1995)
2-Ethyl-1-hexanol	104-76-7	liquid	100%	99	4	7			3.347			58.71			Severe			Balls et al. (1995)
2-Ethyl-1-hexanol	104-76-7	liquid	100%	99	5	7			0.837			19.6			Mild			Balls et al. (1995)
2-Mercaptopyrimidine	1450-85-7	solid	20%	n.p.	1							0			Not Labeled	Not Labeled	Not Labeled	Gautheron et al. (1994)
2-Mercaptopyrimidine	1450-85-7	solid	20%	n.p.	2							-1			Not Labeled			Gautheron et al. (1994)
2-Mercaptopyrimidine	1450-85-7	solid	20%	n.p.	3							-1			Not Labeled			Gautheron et al. (1994)
2-Mercaptopyrimidine	1450-85-7	solid	20%	n.p.	4							1			Not Labeled			Gautheron et al. (1994)
2-Mercaptopyrimidine	1450-85-7	solid	20%	n.p.	5							0			Not Labeled			Gautheron et al. (1994)
2-Mercaptopyrimidine	1450-85-7	solid	20%	n.p.	6							1			Not Labeled			Gautheron et al. (1994)
2-Mercaptopyrimidine	1450-85-7	solid	20%	n.p.	7							0			Not Labeled			Gautheron et al. (1994)
2-Mercaptopyrimidine	1450-85-7	solid	20%	n.p.	8							-8			Not Labeled			Gautheron et al. (1994)
2-Mercaptopyrimidine	1450-85-7	solid	20%	n.p.	9							0			Not Labeled			Gautheron et al. (1994)
2-Mercaptopyrimidine	1450-85-7	solid	20%	n.p.	10							0			Not Labeled			Gautheron et al. (1994)
2-Mercaptopyrimidine	1450-85-7	solid	20%	n.p.	11							-4			Not Labeled			Gautheron et al. (1994)
2-Mercaptopyrimidine	1450-85-7	solid	20%	n.p.	12							-3			Not Labeled			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
2-Methoxyethanol	109-86-4	liquid	100%	n.p.	1							61			Severe	Severe	Cat II	Gautheron et al. (1994)
2-Methoxyethanol	109-86-4	liquid	100%	n.p.	2							69			Severe			Gautheron et al. (1994)
2-Methoxyethanol	109-86-4	liquid	100%	n.p.	3							66			Severe			Gautheron et al. (1994)
2-Methoxyethanol	109-86-4	liquid	100%	n.p.	4							47			Moderate			Gautheron et al. (1994)
2-Methoxyethanol	109-86-4	liquid	100%	n.p.	5							48			Moderate			Gautheron et al. (1994)
2-Methoxyethanol	109-86-4	liquid	100%	n.p.	6							62			Severe			Gautheron et al. (1994)
2-Methoxyethanol	109-86-4	liquid	100%	n.p.	7							65			Severe			Gautheron et al. (1994)
2-Methoxyethanol	109-86-4	liquid	100%	n.p.	8							62			Severe			Gautheron et al. (1994)
2-Methoxyethanol	109-86-4	liquid	100%	n.p.	9							57			Severe			Gautheron et al. (1994)
2-Methoxyethanol	109-86-4	liquid	100%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
2-Methoxyethanol	109-86-4	liquid	100%	n.p.	11							74			Severe			Gautheron et al. (1994)
2-Methoxyethanol	109-86-4	liquid	100%	n.p.	12							88			Severe			Gautheron et al. (1994)
2,2-Dimethylbutanoic acid	595-37-9	liquid	100%	96	1	65			2.583			103.8			Very severe	Very Severe	Cat I	Balls et al. (1995)
2,2-Dimethylbutanoic acid	595-37-9	liquid	100%	96	2	58.3			3.78			115			Very severe			Balls et al. (1995)
2,2-Dimethylbutanoic acid	595-37-9	liquid	100%	96	3	62.7			4.601			131.7			Very severe			Balls et al. (1995)
2,2-Dimethylbutanoic acid	595-37-9	liquid	100%	96	4	84			3.803			130.26			Very severe			Balls et al. (1995)
2,2-Dimethylbutanoic acid	595-37-9	liquid	100%	96	5	37			2.783			78.8			Severe			Balls et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
2,4-Dichloro-5-sulfamoylbenzoic acid	2736-23-4	solid	20%	n.p.	1							23			Mild	Mild	Cat III	Gautheron et al. (1994)
2,4-Dichloro-5-sulfamoylbenzoic acid	2736-23-4	solid	20%	n.p.	2							23			Mild			Gautheron et al. (1994)
2,4-Dichloro-5-sulfamoylbenzoic acid	2736-23-4	solid	20%	n.p.	3							18			Mild			Gautheron et al. (1994)
2,4-Dichloro-5-sulfamoylbenzoic acid	2736-23-4	solid	20%	n.p.	4							28			Moderate			Gautheron et al. (1994)
2,4-Dichloro-5-sulfamoylbenzoic acid	2736-23-4	solid	20%	n.p.	5							16			Mild			Gautheron et al. (1994)
2,4-Dichloro-5-sulfamoylbenzoic acid	2736-23-4	solid	20%	n.p.	6							31			Moderate			Gautheron et al. (1994)
2,4-Dichloro-5-sulfamoylbenzoic acid	2736-23-4	solid	20%	n.p.	7							18			Mild			Gautheron et al. (1994)
2,4-Dichloro-5-sulfamoylbenzoic acid	2736-23-4	solid	20%	n.p.	8							71			Severe			Gautheron et al. (1994)
2,4-Dichloro-5-sulfamoylbenzoic acid	2736-23-4	solid	20%	n.p.	9							19			Mild			Gautheron et al. (1994)
2,4-Dichloro-5-sulfamoylbenzoic acid	2736-23-4	solid	20%	n.p.	10							20			Mild			Gautheron et al. (1994)
2,4-Dichloro-5-sulfamoylbenzoic acid	2736-23-4	solid	20%	n.p.	11							34			Moderate			Gautheron et al. (1994)
2,4-Dichloro-5-sulfamoylbenzoic acid	2736-23-4	solid	20%	n.p.	12							14			Mild			Gautheron et al. (1994)
2,4-Pentanedione	123-54-6	liquid	100%	n.p.	1							61			Severe	Severe	Cat II	Gautheron et al. (1994)
2,4-Pentanedione	123-54-6	liquid	100%	n.p.	2							79			Severe			Gautheron et al. (1994)
2,4-Pentanedione	123-54-6	liquid	100%	n.p.	3							75			Severe			Gautheron et al. (1994)
2,4-Pentanedione	123-54-6	liquid	100%	n.p.	4							34			Moderate			Gautheron et al. (1994)
2,4-Pentanedione	123-54-6	liquid	100%	n.p.	5							70			Severe			Gautheron et al. (1994)
2,4-Pentanedione	123-54-6	liquid	100%	n.p.	6							46			Moderate			Gautheron et al. (1994)
2,4-Pentanedione	123-54-6	liquid	100%	n.p.	7							54			Moderate			Gautheron et al. (1994)
2,4-Pentanedione	123-54-6	liquid	100%	n.p.	8							44			Moderate			Gautheron et al. (1994)
2,4-Pentanedione	123-54-6	liquid	100%	n.p.	9							50			Moderate			Gautheron et al. (1994)
2,4-Pentanedione	123-54-6	liquid	100%	n.p.	10							67			Severe			Gautheron et al. (1994)
2,4-Pentanedione	123-54-6	liquid	100%	n.p.	11							62			Severe			Gautheron et al. (1994)
2,4-Pentanedione	123-54-6	liquid	100%	n.p.	12							76			Severe			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
2,5-Dimethylhexanediol	110-03-2	solid	20%	99.5	1	12			0.415			18.2			Mild	Mild	Cat III	Balls et al. (1995)
2,5-Dimethylhexanediol	110-03-2	solid	20%	99.5	2	10.7			0.979			25.3			Moderate			Balls et al. (1995)
2,5-Dimethylhexanediol	110-03-2	solid	20%	99.5	3	6.7			0.925			20.5			Mild			Balls et al. (1995)
2,5-Dimethylhexanediol	110-03-2	solid	20%	99.5	4	21.33			0.68			31.533			Moderate			Balls et al. (1995)
2,5-Dimethylhexanediol	110-03-2	solid	20%	99.5	5	4.7			0.245			8.3			Mild			Balls et al. (1995)
2,6-Dichlorobenzoyl chloride	4659-45-4	liquid	100%	99	1	9			0.058			9.9			Mild	Mild	Cat III	Balls et al. (1995)
2,6-Dichlorobenzoyl chloride	4659-45-4	liquid	100%	99	2	10.3			0.059			11.2			Mild			Balls et al. (1995)
2,6-Dichlorobenzoyl chloride	4659-45-4	liquid	100%	99	3	9.7			0.078			10.8			Mild			Balls et al. (1995)
2,6-Dichlorobenzoyl chloride	4659-45-4	liquid	100%	99	4	14.33			0.007			14.43			Mild			Balls et al. (1995)
2,6-Dichlorobenzoyl chloride	4659-45-4	liquid	100%	99	5	5.4			0.012			5.6			Mild			Balls et al. (1995)
3-Glycidoxypropyltrimethoxysilane	2530-83-8	liquid	100%	n.p.	1							18			Mild	Mild	Cat III	Gautheron et al. (1994)
3-Glycidoxypropyltrimethoxysilane	2530-83-8	liquid	100%	n.p.	2							24			Mild			Gautheron et al. (1994)
3-Glycidoxypropyltrimethoxysilane	2530-83-8	liquid	100%	n.p.	3							25			Mild			Gautheron et al. (1994)
3-Glycidoxypropyltrimethoxysilane	2530-83-8	liquid	100%	n.p.	4							14			Mild			Gautheron et al. (1994)
3-Glycidoxypropyltrimethoxysilane	2530-83-8	liquid	100%	n.p.	5							13			Mild			Gautheron et al. (1994)
3-Glycidoxypropyltrimethoxysilane	2530-83-8	liquid	100%	n.p.	6							6			Mild			Gautheron et al. (1994)
3-Glycidoxypropyltrimethoxysilane	2530-83-8	liquid	100%	n.p.	7							15			Mild			Gautheron et al. (1994)
3-Glycidoxypropyltrimethoxysilane	2530-83-8	liquid	100%	n.p.	8							18			Mild			Gautheron et al. (1994)
3-Glycidoxypropyltrimethoxysilane	2530-83-8	liquid	100%	n.p.	9							18			Mild			Gautheron et al. (1994)
3-Glycidoxypropyltrimethoxysilane	2530-83-8	liquid	100%	n.p.	10							4			Mild			Gautheron et al. (1994)
3-Glycidoxypropyltrimethoxysilane	2530-83-8	liquid	100%	n.p.	11							23			Mild			Gautheron et al. (1994)
3-Glycidoxypropyltrimethoxysilane	2530-83-8	liquid	100%	n.p.	12							21			Mild			Gautheron et al. (1994)
4-Carboxybenzaldehyde	619-66-9	solid	20%	95	1	97.3			0.02			97.6			Very severe	Severe	Cat II	Balls et al. (1995)
4-Carboxybenzaldehyde	619-66-9	solid	20%	95	2	96.3			0.116			98.1			Very severe			Balls et al. (1995)
4-Carboxybenzaldehyde	619-66-9	solid	20%	95	3	57.3			0.012			57.5			Severe			Balls et al. (1995)
4-Carboxybenzaldehyde	619-66-9	solid	20%	95	4	64			0.022			64.33			Severe			Balls et al. (1995)
4-Carboxybenzaldehyde	619-66-9	solid	20%	95	5	72			0.128			73.9			Severe			Balls et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
4-Carboxybenzaldehyde	619-66-9	solid	20%	95	1 (1)	53.7	3	4.6	0.012	3	0.012	53.9	3	4.9	Moderate	Moderate	Moderate	Southee (1998)
4-Carboxybenzaldehyde	619-66-9	solid	20%	95	1 (2)	47.7	3	3.5	0.002	3	0.02	47.7	3	3.4	Moderate			Southee (1998)
4-Carboxybenzaldehyde	619-66-9	solid	20%	95	2 (1)	46.3	3	3.2	0.05	3	0.021	47.1	3	3.1	Moderate			Southee (1998)
4-Carboxybenzaldehyde	619-66-9	solid	20%	95	2 (2)	46.4	3	2.9	0.058	3	0.014	47.2	3	2.9	Moderate			Southee (1998)
4-Carboxybenzaldehyde	619-66-9	solid	20%	95	3 (1)	42	3	4.5	0.013	3	0.016	42.2	3	4.3	Moderate			Southee (1998)
4-Carboxybenzaldehyde	619-66-9	solid	20%	95	3 (2)	41.3	3	4.0	0.035	3	0.006	41.8	3	3.9	Moderate			Southee (1998)
5-Ethylidene-2-norbornene	16219-75-3	liquid	100%	n.p.	-	5.7			0.207			8.8			Mild	Mild	Cat III	Bailey et al. (2004)
A		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	206.9	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AB		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	90	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AC		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	134.8	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
Acetone	67-64-1	liquid	100%	99	1	90.3			3.676			145.5			Very severe	Very Severe	Cat I	Balls et al. (1995)
Acetone	67-64-1	liquid	100%	99	2	83.7			2.389			119.5			Very severe			Balls et al. (1995)
Acetone	67-64-1	liquid	100%	99	3	55.7			4.315			120.4			Very severe			Balls et al. (1995)
Acetone	67-64-1	liquid	100%	99	4	94.33			2.492			131.72			Very severe			Balls et al. (1995)
Acetone	67-64-1	liquid	100%	99	5	69.3			1.942			98.4			Very severe			Balls et al. (1995)
AD		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	113.1	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AE		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	66.7	n.p.	n.p.	Cat I	Cat I	Cat II	AMCP BRD
AF		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	9.6	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD
AG		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	391.9	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AH		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	255.7	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AI		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	354.7	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AJ		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	357.1	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AK		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	444.3	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AL		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	353.6	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
Alkyl phosphoric acid ester/amine salt	-	liquid	100%	n.p.	-	37.7			3.577			91.3			Severe	Severe	Cat I	Bailey et al. (2004)
All Purpose Cleaner (#5)	-	liquid	100%	n.p.	-	102.5	5		1.252	5		121.3	5		Severe	Severe	Cat I	Swanson et al. (1995)
All Purpose Cleaner (#7)	-	liquid	100%	n.p.	-	348.1	5		3.013	5		393.3	5		Severe	Severe	Cat I	Swanson et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Allyl alcohol	107-18-6	liquid	100%	n.p.	1							156			Severe	Severe	Cat I	Gautheron et al. (1994)
Allyl alcohol	107-18-6	liquid	100%	n.p.	2							138			Severe			Gautheron et al. (1994)
Allyl alcohol	107-18-6	liquid	100%	n.p.	3							232			Severe			Gautheron et al. (1994)
Allyl alcohol	107-18-6	liquid	100%	n.p.	4							156			Severe			Gautheron et al. (1994)
Allyl alcohol	107-18-6	liquid	100%	n.p.	5							132			Severe			Gautheron et al. (1994)
Allyl alcohol	107-18-6	liquid	100%	n.p.	6							191			Severe			Gautheron et al. (1994)
Allyl alcohol	107-18-6	liquid	100%	n.p.	7							190			Severe			Gautheron et al. (1994)
Allyl alcohol	107-18-6	liquid	100%	n.p.	8							166			Severe			Gautheron et al. (1994)
Allyl alcohol	107-18-6	liquid	100%	n.p.	9							123			Severe			Gautheron et al. (1994)
Allyl alcohol	107-18-6	liquid	100%	n.p.	10							101			Severe			Gautheron et al. (1994)
Allyl alcohol	107-18-6	liquid	100%	n.p.	11							200			Severe			Gautheron et al. (1994)
Allyl alcohol	107-18-6	liquid	100%	n.p.	12							90			Severe			Gautheron et al. (1994)
Aluminum hydroxide	21645-51-2	solid	20%	n.p.	1							5			Mild	Mild	Cat III	Gautheron et al. (1994)
Aluminum hydroxide	21645-51-2	solid	20%	n.p.	2							4			Mild			Gautheron et al. (1994)
Aluminum hydroxide	21645-51-2	solid	20%	n.p.	3							10			Mild			Gautheron et al. (1994)
Aluminum hydroxide	21645-51-2	solid	20%	n.p.	4							3			Not Labeled			Gautheron et al. (1994)
Aluminum hydroxide	21645-51-2	solid	20%	n.p.	5							5			Mild			Gautheron et al. (1994)
Aluminum hydroxide	21645-51-2	solid	20%	n.p.	6							28			Moderate			Gautheron et al. (1994)
Aluminum hydroxide	21645-51-2	solid	20%	n.p.	7							2			Not Labeled			Gautheron et al. (1994)
Aluminum hydroxide	21645-51-2	solid	20%	n.p.	8							4			Mild			Gautheron et al. (1994)
Aluminum hydroxide	21645-51-2	solid	20%	n.p.	9							10			Mild			Gautheron et al. (1994)
Aluminum hydroxide	21645-51-2	solid	20%	n.p.	10							6			Mild			Gautheron et al. (1994)
Aluminum hydroxide	21645-51-2	solid	20%	n.p.	11							2			Not Labeled			Gautheron et al. (1994)
Aluminum hydroxide	21645-51-2	solid	20%	n.p.	12							2			Not Labeled			Gautheron et al. (1994)
AM		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	135.8	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Ammonium nitrate	6484-52-2	solid	20%	>99.9	1	6.3			0.132			8.3			Mild	Mild	Cat III	Balls et al. (1995)
Ammonium nitrate	6484-52-2	solid	20%	>99.9	2	6			0.026			6.4			Mild			Balls et al. (1995)
Ammonium nitrate	6484-52-2	solid	20%	>99.9	3	6			0.079			7.2			Mild			Balls et al. (1995)
Ammonium nitrate	6484-52-2	solid	20%	>99.9	4	11.34			0.698			21.82			Mild			Balls et al. (1995)
Ammonium nitrate	6484-52-2	solid	20%	>99.9	5	4.7			0.034			5.2			Mild			Balls et al. (1995)
Ammonium nitrate	6484-52-2	n.p.	100%	n.p.	1 (1)	4.3	3	2.1	0.037	3	0.036	4.9	3	2.4	Mild	Mild	Mild	Southee (1998)
Ammonium nitrate	6484-52-2	n.p.	100%	n.p.	1 (2)	5.0	3	1.2	0.059	3	0.031	5.9	3	1.4	Mild			Southee (1998)
Ammonium nitrate	6484-52-2	n.p.	100%	n.p.	2 (1)	1.6	3	1.2	0.153	3	0.059	3.9	3	1.8	Mild			Southee (1998)
Ammonium nitrate	6484-52-2	n.p.	100%	n.p.	2 (2)	2.0	3	0.6	0.107	3	0.044	3.6	3	1	Mild			Southee (1998)
Ammonium nitrate	6484-52-2	n.p.	100%	n.p.	3 (1)	3.7	3	0.6	0.100	3	0.033	5.2	3	0.6	Mild			Southee (1998)
Ammonium nitrate	6484-52-2	n.p.	100%	n.p.	3 (2)	4.3	3	0.6	0.158	3	0.07	6.7	3	1.5	Mild			Southee (1998)
AN		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	113.5	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
Anthracene	120-12-7	solid	20%	n.p.	1							-2			Not Labeled	Not Labeled	Not Labeled	Gautheron et al. (1994)
Anthracene	120-12-7	solid	20%	n.p.	2							1			Not Labeled			Gautheron et al. (1994)
Anthracene	120-12-7	solid	20%	n.p.	3							-3			Not Labeled			Gautheron et al. (1994)
Anthracene	120-12-7	solid	20%	n.p.	4							0			Not Labeled			Gautheron et al. (1994)
Anthracene	120-12-7	solid	20%	n.p.	5							0			Not Labeled			Gautheron et al. (1994)
Anthracene	120-12-7	solid	20%	n.p.	6							-1			Not Labeled			Gautheron et al. (1994)
Anthracene	120-12-7	solid	20%	n.p.	7							0			Not Labeled			Gautheron et al. (1994)
Anthracene	120-12-7	solid	20%	n.p.	8							0			Not Labeled			Gautheron et al. (1994)
Anthracene	120-12-7	solid	20%	n.p.	9							1			Not Labeled			Gautheron et al. (1994)
Anthracene	120-12-7	solid	20%	n.p.	10							0			Not Labeled			Gautheron et al. (1994)
Anthracene	120-12-7	solid	20%	n.p.	11							-2			Not Labeled			Gautheron et al. (1994)
Anthracene	120-12-7	solid	20%	n.p.	12							2			Not Labeled			Gautheron et al. (1994)
AO		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	216.2	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AP		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	393.3	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AQ		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	84.9	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AR		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	116.1	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Aromatic hydrocarbon #1	-	liquid	100%	n.p.	-	2.7			0.000			2.7			Not Labeled	Not Labeled	Not Labeled	Bailey et al. (2004)
Aromatic hydrocarbon #2	-	liquid	100%	n.p.	-	4.3			0.017			4.6			Mild	Mild	Cat III	Bailey et al. (2004)
Aryl phosponates	-	liquid	100%	n.p.	-	20.3			1.399			41.3			Moderate	Moderate	Cat II	Bailey et al. (2004)
AS		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	79.8	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AT		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	85.6	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AU		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	122.2	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AV		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	191.8	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AW		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	43.1	n.p.	n.p.	Cat II	Cat II	Cat II	AMCP BRD
AX		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	157.3	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
AY		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	194.3	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
B		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	152.2	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
Bathroom Cleaner (#6)	-	liquid	100%	n.p.	-	64	5		0.95	5		78.3	5		Severe	Severe	Cat I	Swanson et al. (1995)
BB		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	2	n.p.	n.p.	Not Labeled	Not Labeled	Cat IV	AMCP BRD
BD		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	18.3	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD
BE		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	15	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD
Benchmark-Group 1 (#12)	-	liquid	100%	n.p.	-							60.1			Severe	Severe	Cat II	Swanson and Harbell (2000)
Benchmark-Group 2 (#13)	-	liquid	100%	n.p.	-							60.1			Severe	Severe	Cat II	Swanson and Harbell (2000)
Benzalkonium chloride (1 %)	8001-54-5	liquid	1%	98	1	59			3.588			112.8			Very severe	Very Severe	Cat I	Balls et al. (1995)
Benzalkonium chloride (1 %)	8001-54-5	liquid	1%	98	2	37			3.566			90.5			Very severe			Balls et al. (1995)
Benzalkonium chloride (1 %)	8001-54-5	liquid	1%	98	3	34.3			4.336			99.4			Very severe			Balls et al. (1995)
Benzalkonium chloride (1 %)	8001-54-5	liquid	1%	98	4	22			2.699			62.49			Severe			Balls et al. (1995)
Benzalkonium chloride (1 %)	8001-54-5	liquid	1%	98	5	38			2.706			78.6			Severe			Balls et al. (1995)
Benzalkonium chloride (10%)	8001-54-5	liquid	10%	98	1	75.3			4.456			142.2			Very severe	Very Severe	Cat I	Balls et al. (1995)
Benzalkonium chloride (10%)	8001-54-5	liquid	10%	98	2	79.3			5.223			157.7			Very severe			Balls et al. (1995)
Benzalkonium chloride (10%)	8001-54-5	liquid	10%	98	3	61.7			4.142			123.8			Very severe			Balls et al. (1995)
Benzalkonium chloride (10%)	8001-54-5	liquid	10%	98	4	63			4.967			137.5			Very severe			Balls et al. (1995)
Benzalkonium chloride (10%)	8001-54-5	liquid	10%	98	5	74.7			3.096			121.1			Very severe			Balls et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	1 (1)	84.0	3	3.8	7.408	3	0.903	195.2	3	11.3	Very severe	Very Severe	Cat I	Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	1 (2)	85.6	3	3.2	3.305	3	0.225	135.2	3	5.2	Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	1 (3)	82.0	3	1.7	3.729	3	0.25	137.9	3	2.3	Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	1 (4)	85.0	3	5.2	4.766	3	1.132	156.5	3	18.6	Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	1 (5)	87.7	3	1.7	3.354	3	0.108	138.0	3	0.1	Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	1 (6)	91.7	3	7.0	5.67	3	1.096	176.8			Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	1 (7)	98.3	3	2.6	5.645	3	0.523	183.0			Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	1 (8)	87.7	3	2.9	5.848	3	0.581	175.4			Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	2 (1)	88.0	3	7.5	4.426	3	0.623	154.4	3	11.1	Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	2 (2)	94.6	3	10.4	4.148	3	0.662	156.9	3	18.6	Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	2 (3)	87.0	3	7.5	4.252	3	0.069	150.8	3	7.1	Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	2 (4)	93.0	3	3.0	4.278	3	1.058	157.2	3	18.0	Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	2 (5)	98.3	3	2.3	3.972	3	0.360	157.9	3	3.4	Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	2 (6)	95.7	3	5.0	4.129	3	0.581	157.0			Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	2 (7)	98.0	3	5.1	4.144	3	0.232	160.2			Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	3 (1)	96.7	3	2.0	4.015	3	1.011	156.9	3	17.1	Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	3 (2)	92.6	3	11.8	4.719	3	1.547	163.4	3	16.2	Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	3 (3)	105.0	3	6.1	4.316	3	0.320	169.7	3	10.2	Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	3 (4)	95.3	3	4.0	4.497	3	1.007	162.8	3	11.4	Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	3 (5)	92.3	3	7.2	3.948	3	0.231	151.6	3	7.7	Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	3 (6)	93.7	3	4.9	4.624	3	1.708	163.1	3	22.1	Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	3 (7)	100.7	3	2.5	4.473	3	0.619	167.8	3	7.8	Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	3 (8)	96.7	3	2.0	9.016	3	1.011	156.9	3	17.1	Very severe			Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	3 (9)	97.3	3	5.1	4.183	3	0.514	160.0	3	8.2	Very severe			Southee (1998)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Benzalkonium chloride (5%)	8001-54-5	liquid	5%	98	1	126.6			3.264			126.6			Very severe	Very Severe	Cat I	Balls et al. (1995)
Benzalkonium chloride (5%)	8001-54-5	liquid	5%	98	2	163.7			6.599			163.7			Very severe			Balls et al. (1995)
Benzalkonium chloride (5%)	8001-54-5	liquid	5%	98	3	110.7			3.891			110.7			Very severe			Balls et al. (1995)
Benzalkonium chloride (5%)	8001-54-5	liquid	5%	98	4	130.41			4.338			130.41			Very severe			Balls et al. (1995)
Benzalkonium chloride (5%)	8001-54-5	liquid	5%	98	5	111.1			3.117			111.1			Very severe			Balls et al. (1995)
Benzethonium chloride	121-54-0	surfactant	10%	n.p.	1							128			Severe	Severe	Cat I	Gautheron et al. (1994)
Benzethonium chloride	121-54-0	surfactant	10%	n.p.	2							124			Severe			Gautheron et al. (1994)
Benzethonium chloride	121-54-0	surfactant	10%	n.p.	3							163			Severe			Gautheron et al. (1994)
Benzethonium chloride	121-54-0	surfactant	10%	n.p.	4							106			Severe			Gautheron et al. (1994)
Benzethonium chloride	121-54-0	surfactant	10%	n.p.	5							128			Severe			Gautheron et al. (1994)
Benzethonium chloride	121-54-0	surfactant	10%	n.p.	6							129			Severe			Gautheron et al. (1994)
Benzethonium chloride	121-54-0	surfactant	10%	n.p.	7							142			Severe			Gautheron et al. (1994)
Benzethonium chloride	121-54-0	surfactant	10%	n.p.	8							129			Severe			Gautheron et al. (1994)
Benzethonium chloride	121-54-0	surfactant	10%	n.p.	9							166			Severe			Gautheron et al. (1994)
Benzethonium chloride	121-54-0	surfactant	10%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Benzethonium chloride	121-54-0	surfactant	10%	n.p.	11							142			Severe			Gautheron et al. (1994)
Benzethonium chloride	121-54-0	surfactant	10%	n.p.	12							116			Severe			Gautheron et al. (1994)
Benzoyl-L-tartaric acid	2743-38-6	solid	20%													Severe	Cat I	Balls et al. (1995)
Benzoyl-L-tartaric acid	2743-38-6	solid	20%															Balls et al. (1995)
Benzoyl-L-tartaric acid	2743-38-6	solid	20%															Balls et al. (1995)
Benzoyl-L-tartaric acid	2743-38-6	solid	20%															Balls et al. (1995)
Benzoyl-L-tartaric acid	2743-38-6	solid	20%															Balls et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Betaine monohydrate	590-47-6	solid	20%	n.p.	1							4			Mild	Mild	Cat III	Gautheron et al. (1994)
Betaine monohydrate	590-47-6	solid	20%	n.p.	2							1			Not Labeled			Gautheron et al. (1994)
Betaine monohydrate	590-47-6	solid	20%	n.p.	3							0			Not Labeled			Gautheron et al. (1994)
Betaine monohydrate	590-47-6	solid	20%	n.p.	4							1			Not Labeled			Gautheron et al. (1994)
Betaine monohydrate	590-47-6	solid	20%	n.p.	5							1			Not Labeled			Gautheron et al. (1994)
Betaine monohydrate	590-47-6	solid	20%	n.p.	6							3			Not Labeled			Gautheron et al. (1994)
Betaine monohydrate	590-47-6	solid	20%	n.p.	7							1			Not Labeled			Gautheron et al. (1994)
Betaine monohydrate	590-47-6	solid	20%	n.p.	8							-10			Not Labeled			Gautheron et al. (1994)
Betaine monohydrate	590-47-6	solid	20%	n.p.	9							4			Mild			Gautheron et al. (1994)
Betaine monohydrate	590-47-6	solid	20%	n.p.	10							-1			Not Labeled			Gautheron et al. (1994)
Betaine monohydrate	590-47-6	solid	20%	n.p.	11							1			Not Labeled			Gautheron et al. (1994)
Betaine monohydrate	590-47-6	solid	20%	n.p.	12							6			Mild			Gautheron et al. (1994)
BF		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	63.5	n.p.	n.p.	Cat I	Cat I	Cat II	AMCP BRD
BJ		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	78.3	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
BJ		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	54.6	n.p.	n.p.	Cat II	Cat II	Cat II	AMCP BRD
BK		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	6.7	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD
BL		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	6	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD
BM		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	25.4	n.p.	n.p.	Cat II	Cat II	Cat II	AMCP BRD
BN		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	13.5	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD
BP		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	19.1	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD
BQ		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	33.6	n.p.	n.p.	Cat II	Cat II	Cat II	AMCP BRD

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
BRIJ-35	9002-92-0	surfactant	10%	n.p.	1							0			Not Labeled	Not Labeled	Cat IV	Gautheron et al. (1994)
BRIJ-35	9002-92-0	surfactant	10%	n.p.	2							2			Not Labeled			Gautheron et al. (1994)
BRIJ-35	9002-92-0	surfactant	10%	n.p.	3							-1			Not Labeled			Gautheron et al. (1994)
BRIJ-35	9002-92-0	surfactant	10%	n.p.	4							1			Not Labeled			Gautheron et al. (1994)
BRIJ-35	9002-92-0	surfactant	10%	n.p.	5							0			Not Labeled			Gautheron et al. (1994)
BRIJ-35	9002-92-0	surfactant	10%	n.p.	6							0			Not Labeled			Gautheron et al. (1994)
BRIJ-35	9002-92-0	surfactant	10%	n.p.	7							0			Not Labeled			Gautheron et al. (1994)
BRIJ-35	9002-92-0	surfactant	10%	n.p.	8							0			Not Labeled			Gautheron et al. (1994)
BRIJ-35	9002-92-0	surfactant	10%	n.p.	9							1			Not Labeled			Gautheron et al. (1994)
BRIJ-35	9002-92-0	surfactant	10%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
BRIJ-35	9002-92-0	surfactant	10%	n.p.	11							-2			Not Labeled			Gautheron et al. (1994)
BRIJ-35	9002-92-0	surfactant	10%	n.p.	12							-2			Not Labeled			Gautheron et al. (1994)
Butyl acetate	123-86-4	liquid	100%	99	1	9			2.7			49.5			Moderate	Moderate	Cat II	Balls et al. (1995)
Butyl acetate	123-86-4	liquid	100%	99	2	7.7			1.989			37.5			Moderate			Balls et al. (1995)
Butyl acetate	123-86-4	liquid	100%	99	3	5.7			2.546			43.9			Moderate			Balls et al. (1995)
Butyl acetate	123-86-4	liquid	100%	99	4	5			1.257			23.86			Mild			Balls et al. (1995)
Butyl acetate	123-86-4	liquid	100%	99	5	2.3			1.051			18.1			Mild			Balls et al. (1995)
Butyl cellusolve	111-76-2	liquid	100%	n.p.	1 (1)	39	3	7.8	4.625	3	0.471	108.3	3	12.9	Very severe	Very Severe	Cat I	Southee (1998)
Butyl cellusolve	111-76-2	liquid	100%	n.p.	1 (2)	43	3	4.0	4.589	3	0.418	111.8	3	5.5	Very severe			Southee (1998)
Butyl cellusolve	111-76-2	liquid	100%	n.p.	2 (1)	29.6	3	1.5	4.213	3	0.78	92.8	3	13	Very severe			Southee (1998)
Butyl cellusolve	111-76-2	liquid	100%	n.p.	2 (2)	31.3	3	2.3	4.526	3	0.864	99.2	3	10.7	Very severe			Southee (1998)
Butyl cellusolve	111-76-2	liquid	100%	n.p.	3 (1)	37.7	3	1.0	3.813	3	0.933	94.9	3	13.8	Very severe			Southee (1998)
Butyl cellusolve	111-76-2	liquid	100%	n.p.	3 (2)	37.7	3	6.1	4.031	3	1.206	98.2	3	21.6	Very severe			Southee (1998)
Butyrolactone	96-48-0	liquid	100%	n.p.	1							48			Moderate			Gautheron et al. (1994)
Butyrolactone	96-48-0	liquid	100%	n.p.	2							44			Moderate			Gautheron et al. (1994)
Butyrolactone	96-48-0	liquid	100%	n.p.	3							64			Severe			Gautheron et al. (1994)
Butyrolactone	96-48-0	liquid	100%	n.p.	4							35			Moderate			Gautheron et al. (1994)
Butyrolactone	96-48-0	liquid	100%	n.p.	5							35			Moderate			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Butyrolactone	96-48-0	liquid	100%	n.p.	6							30			Moderate	Moderate	Cat II	Gautheron et al. (1994)
Butyrolactone	96-48-0	liquid	100%	n.p.	7							80			Severe			Gautheron et al. (1994)
Butyrolactone	96-48-0	liquid	100%	n.p.	8							32			Moderate			Gautheron et al. (1994)
Butyrolactone	96-48-0	liquid	100%	n.p.	9							42			Moderate			Gautheron et al. (1994)
Butyrolactone	96-48-0	liquid	100%	n.p.	10							53			Moderate			Gautheron et al. (1994)
Butyrolactone	96-48-0	liquid	100%	n.p.	11							35			Moderate			Gautheron et al. (1994)
Butyrolactone	96-48-0	liquid	100%	n.p.	12							49			Moderate			Gautheron et al. (1994)
C		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	29.7	n.p.	n.p.	Cat II	Cat II	Cat II	AMCP BRD
Captan 90 concentrate	133-06-2	solid	20%	90	1	28			-0.008			27.8			Moderate	Moderate	Cat II	Balls et al. (1995)
Captan 90 concentrate	133-06-2	solid	20%	90	2	26.3			0.055			27.2			Moderate			Balls et al. (1995)
Captan 90 concentrate	133-06-2	solid	20%	90	3	34.7			0.007			34.8			Moderate			Balls et al. (1995)
Captan 90 concentrate	133-06-2	solid	20%	90	4	102			0.061			102.918			Very severe			Balls et al. (1995)
Captan 90 concentrate	133-06-2	solid	20%	90	5	26.3			0.004			26.4			Moderate			Balls et al. (1995)
Carboxylic acid amides	-	solid	100%	n.p.	-	10.7			1.125			27.5			Moderate	Moderate	Cat II	Bailey et al. (2004)
Cetylpyridinium bromide (0.1%)	140-72-7	liquid	0.10%	98	1	6.7			0.293			11			Mild	Mild	Cat III	Balls et al. (1995)
Cetylpyridinium bromide (0.1%)	140-72-7	liquid	0.10%	98	2	1.7			0.163			4.1			Mild			Balls et al. (1995)
Cetylpyridinium bromide (0.1%)	140-72-7	liquid	0.10%	98	3	3			0.606			12.1			Mild			Balls et al. (1995)
Cetylpyridinium bromide (0.1%)	140-72-7	liquid	0.10%	98	4	3.33			0.066			4.33			Mild			Balls et al. (1995)
Cetylpyridinium bromide (0.1%)	140-72-7	liquid	0.10%	98	5	6.3			0.543			14.5			Mild			Balls et al. (1995)
Cetylpyridinium bromide (10%)	140-72-7	liquid	10%	98	1	22.7			1.389			43.5			Moderate	Very Severe	Cat I	Balls et al. (1995)
Cetylpyridinium bromide (10%)	140-72-7	liquid	10%	98	2	27.7			4.128			89.6			Very severe			Balls et al. (1995)
Cetylpyridinium bromide (10%)	140-72-7	liquid	10%	98	3	24.7			3.759			81			Very severe			Balls et al. (1995)
Cetylpyridinium bromide (10%)	140-72-7	liquid	10%	98	4	17			3.97			71.22			Severe			Balls et al. (1995)
Cetylpyridinium bromide (10%)	140-72-7	liquid	10%	98	5	23			3.58			76.7			Severe			Balls et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Cetylpyridinium bromide (6%)	140-72-7	liquid	6%	98	1	31.7			2.705			72.2			Severe	Severe	Cat II	Balls et al. (1995)
Cetylpyridinium bromide (6%)	140-72-7	liquid	6%	98	2	38.3			3.195			86.3			Very severe			Balls et al. (1995)
Cetylpyridinium bromide (6%)	140-72-7	liquid	6%	98	3	18.3			3.015			63.6			Severe			Balls et al. (1995)
Cetylpyridinium bromide (6%)	140-72-7	liquid	6%	98	4	25.33			2.892			68.72			Severe			Balls et al. (1995)
Cetylpyridinium bromide (6%)	140-72-7	liquid	6%	98	5	34			2.097			65.4			Severe			Balls et al. (1995)
CG		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	3.9	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD
CH		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	17.4	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD
Chlorhexidine	55-56-1	solid	20%	n.p.[12]	1	141			0.399			147			Very severe	Very Severe	Cat I	Balls et al. (1995)
Chlorhexidine	55-56-1	solid	20%	n.p.	2	124			-0.071			122.9			Very severe			Balls et al. (1995)
Chlorhexidine	55-56-1	solid	20%	n.p.	3	96.3			0.062			97.3			Very severe			Balls et al. (1995)
Chlorhexidine	55-56-1	solid	20%	n.p.	4	97.66			0.277			101.78			Very severe			Balls et al. (1995)
Chlorhexidine	55-56-1	solid	20%	n.p.	5	98.7			0.189			101.5			Very severe			Balls et al. (1995)
Clarified slurry oil	-	liquid	100%	n.p.	-	2.3			0.000			2.3			Not Labeled	Not Labeled	Cat IV	Bailey et al. (2004)
Cleaner/Degreaser (#13)	-	liquid	100%	n.p.	-	314.3	5		2.623	5		353.6	5		Severe	Severe	Cat I	Swanson et al. (1995)
Cutting fluid (conc.) #1	-	liquid	100%	n.p.	-	3.3			0.001			3.5			Mild	Mild	Cat III	Bailey et al. (2004)
Cutting fluid (conc.) #2	-	liquid	100%	n.p.	-	4.3			0.038			4.9			Mild	Mild	Cat III	Bailey et al. (2004)
Cyclohexanol	108-93-0	liquid	100%	97	1	18.3			4.442			85			Very severe	Moderate	Cat II	Balls et al. (1995)
Cyclohexanol	108-93-0	liquid	100%	97	2	7.3			2.838			49.9			Moderate			Balls et al. (1995)
Cyclohexanol	108-93-0	liquid	100%	97	3	12			3.87			70.1			Severe			Balls et al. (1995)
Cyclohexanol	108-93-0	liquid	100%	97	4	11.66			2.71			52.24			Moderate			Balls et al. (1995)
Cyclohexanol	108-93-0	liquid	100%	97	5	7			2.392			43.2			Moderate			Balls et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Cyclohexanone	108-94-1	liquid	100%	n.p.	1							92			Severe	Severe	Cat I	Gautheron et al. (1994)
Cyclohexanone	108-94-1	liquid	100%	n.p.	2							108			Severe			Gautheron et al. (1994)
Cyclohexanone	108-94-1	liquid	100%	n.p.	3							96			Severe			Gautheron et al. (1994)
Cyclohexanone	108-94-1	liquid	100%	n.p.	4							81			Severe			Gautheron et al. (1994)
Cyclohexanone	108-94-1	liquid	100%	n.p.	5							130			Severe			Gautheron et al. (1994)
Cyclohexanone	108-94-1	liquid	100%	n.p.	6							93			Severe			Gautheron et al. (1994)
Cyclohexanone	108-94-1	liquid	100%	n.p.	7							104			Severe			Gautheron et al. (1994)
Cyclohexanone	108-94-1	liquid	100%	n.p.	8							90			Severe			Gautheron et al. (1994)
Cyclohexanone	108-94-1	liquid	100%	n.p.	9							142			Severe			Gautheron et al. (1994)
Cyclohexanone	108-94-1	liquid	100%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Cyclohexanone	108-94-1	liquid	100%	n.p.	11							118			Severe			Gautheron et al. (1994)
Cyclohexanone	108-94-1	liquid	100%	n.p.	12							108			Severe			Gautheron et al. (1994)
D		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	187.7	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
Degreaser (#16)	-	liquid	100%	n.p.	-	225.4	5		2.022	5		255.7	5		Severe	Severe	Cat I	Swanson et al. (1995)
Deoxycholic acid, sodium salt	302-95-4	surfactant	10%	n.p.	1							96			Severe	Severe	Cat I	Gautheron et al. (1994)
Deoxycholic acid, sodium salt	302-95-4	surfactant	10%	n.p.	2							72			Severe			Gautheron et al. (1994)
Deoxycholic acid, sodium salt	302-95-4	surfactant	10%	n.p.	3							106			Severe			Gautheron et al. (1994)
Deoxycholic acid, sodium salt	302-95-4	surfactant	10%	n.p.	4							73			Severe			Gautheron et al. (1994)
Deoxycholic acid, sodium salt	302-95-4	surfactant	10%	n.p.	5							119			Severe			Gautheron et al. (1994)
Deoxycholic acid, sodium salt	302-95-4	surfactant	10%	n.p.	6							103			Severe			Gautheron et al. (1994)
Deoxycholic acid, sodium salt	302-95-4	surfactant	10%	n.p.	7							88			Severe			Gautheron et al. (1994)
Deoxycholic acid, sodium salt	302-95-4	surfactant	10%	n.p.	8							46			Moderate			Gautheron et al. (1994)
Deoxycholic acid, sodium salt	302-95-4	surfactant	10%	n.p.	9							100			Severe			Gautheron et al. (1994)
Deoxycholic acid, sodium salt	302-95-4	surfactant	10%	n.p.	10							60			Severe			Gautheron et al. (1994)
Deoxycholic acid, sodium salt	302-95-4	surfactant	10%	n.p.	11							200			Severe			Gautheron et al. (1994)
Deoxycholic acid, sodium salt	302-95-4	surfactant	10%	n.p.	12							59			Severe			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Diacetone alcohol	123-42-2	liquid	100%	n.p.	1							53			Moderate	Moderate	Cat II	Gautheron et al. (1994)
Diacetone alcohol	123-42-2	liquid	100%	n.p.	2							41			Moderate			Gautheron et al. (1994)
Diacetone alcohol	123-42-2	liquid	100%	n.p.	3							105			Severe			Gautheron et al. (1994)
Diacetone alcohol	123-42-2	liquid	100%	n.p.	4							39			Moderate			Gautheron et al. (1994)
Diacetone alcohol	123-42-2	liquid	100%	n.p.	5							42			Moderate			Gautheron et al. (1994)
Diacetone alcohol	123-42-2	liquid	100%	n.p.	6							34			Moderate			Gautheron et al. (1994)
Diacetone alcohol	123-42-2	liquid	100%	n.p.	7							49			Moderate			Gautheron et al. (1994)
Diacetone alcohol	123-42-2	liquid	100%	n.p.	8							41			Moderate			Gautheron et al. (1994)
Diacetone alcohol	123-42-2	liquid	100%	n.p.	9							92			Severe			Gautheron et al. (1994)
Diacetone alcohol	123-42-2	liquid	100%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Diacetone alcohol	123-42-2	liquid	100%	n.p.	11							36			Moderate			Gautheron et al. (1994)
Diacetone alcohol	123-42-2	liquid	100%	n.p.	12							56			Severe			Gautheron et al. (1994)
Dibenzoyl-L-tartaric acid	2743-38-6	solid	20%	n.p.	1							104			Severe	Severe	Cat I	Gautheron et al. (1994)
Dibenzoyl-L-tartaric acid	2743-38-6	solid	20%	n.p.	2							134			Severe			Gautheron et al. (1994)
Dibenzoyl-L-tartaric acid	2743-38-6	solid	20%	n.p.	3							82			Severe			Gautheron et al. (1994)
Dibenzoyl-L-tartaric acid	2743-38-6	solid	20%	n.p.	4							118			Severe			Gautheron et al. (1994)
Dibenzoyl-L-tartaric acid	2743-38-6	solid	20%	n.p.	5							110			Severe			Gautheron et al. (1994)
Dibenzoyl-L-tartaric acid	2743-38-6	solid	20%	n.p.	6							66			Severe			Gautheron et al. (1994)
Dibenzoyl-L-tartaric acid	2743-38-6	solid	20%	n.p.	7							88			Severe			Gautheron et al. (1994)
Dibenzoyl-L-tartaric acid	2743-38-6	solid	20%	n.p.	8							193			Severe			Gautheron et al. (1994)
Dibenzoyl-L-tartaric acid	2743-38-6	solid	20%	n.p.	9							82			Severe			Gautheron et al. (1994)
Dibenzoyl-L-tartaric acid	2743-38-6	solid	20%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Dibenzoyl-L-tartaric acid	2743-38-6	solid	20%	n.p.	11							213			Severe			Gautheron et al. (1994)
Dibenzoyl-L-tartaric acid	2743-38-6	solid	20%	n.p.	12							135			Severe			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Dibenzyl phosphate	1623-08-1	solid	20%	99	1	304.3			-0.017			304.1			Very severe	Very Severe	Cat I	Balls et al. (1995)
Dibenzyl phosphate	1623-08-1	solid	20%	99	2	389.3			0.117			391.1			Very severe			Balls et al. (1995)
Dibenzyl phosphate	1623-08-1	solid	20%	99	3	418			-0.002			418			Very severe			Balls et al. (1995)
Dibenzyl phosphate	1623-08-1	solid	20%	99	4	467			-0.016			467.09			Very severe			Balls et al. (1995)
Dibenzyl phosphate	1623-08-1	solid	20%	99	5	304			0.234			307.5			Very severe			Balls et al. (1995)
Dimethyl sulfoxide	67-68-5	liquid	100%	n.p.	1							10			Mild	Mild	Cat III	Gautheron et al. (1994)
Dimethyl sulfoxide	67-68-5	liquid	100%	n.p.	2							10			Mild			Gautheron et al. (1994)
Dimethyl sulfoxide	67-68-5	liquid	100%	n.p.	3							14			Mild			Gautheron et al. (1994)
Dimethyl sulfoxide	67-68-5	liquid	100%	n.p.	4							11			Mild			Gautheron et al. (1994)
Dimethyl sulfoxide	67-68-5	liquid	100%	n.p.	5							11			Mild			Gautheron et al. (1994)
Dimethyl sulfoxide	67-68-5	liquid	100%	n.p.	6							14			Mild			Gautheron et al. (1994)
Dimethyl sulfoxide	67-68-5	liquid	100%	n.p.	7							10			Mild			Gautheron et al. (1994)
Dimethyl sulfoxide	67-68-5	liquid	100%	n.p.	8							10			Mild			Gautheron et al. (1994)
Dimethyl sulfoxide	67-68-5	liquid	100%	n.p.	9							9			Mild			Gautheron et al. (1994)
Dimethyl sulfoxide	67-68-5	liquid	100%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Dimethyl sulfoxide	67-68-5	liquid	100%	n.p.	11							4			Mild			Gautheron et al. (1994)
Dimethyl sulfoxide	67-68-5	liquid	100%	n.p.	12							22			Mild			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Dimethylbiguanide	657-24-9	solid	20%	n.p.	1							0			Not Labeled	Not Labeled	Cat IV	Gautheron et al. (1994)
Dimethylbiguanide	657-24-9	solid	20%	n.p.	2							3			Not Labeled			Gautheron et al. (1994)
Dimethylbiguanide	657-24-9	solid	20%	n.p.	3							1			Not Labeled			Gautheron et al. (1994)
Dimethylbiguanide	657-24-9	solid	20%	n.p.	4							3			Not Labeled			Gautheron et al. (1994)
Dimethylbiguanide	657-24-9	solid	20%	n.p.	5							1			Not Labeled			Gautheron et al. (1994)
Dimethylbiguanide	657-24-9	solid	20%	n.p.	6							5			Mild			Gautheron et al. (1994)
Dimethylbiguanide	657-24-9	solid	20%	n.p.	7							3			Not Labeled			Gautheron et al. (1994)
Dimethylbiguanide	657-24-9	solid	20%	n.p.	8							1			Not Labeled			Gautheron et al. (1994)
Dimethylbiguanide	657-24-9	solid	20%	n.p.	9							2			Not Labeled			Gautheron et al. (1994)
Dimethylbiguanide	657-24-9	solid	20%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Dimethylbiguanide	657-24-9	solid	20%	n.p.	11							5			Mild			Gautheron et al. (1994)
Dimethylbiguanide	657-24-9	solid	20%	n.p.	12							8			Mild			Gautheron et al. (1994)
DL-Glutamic acid	19285-83-7	solid	20%	n.p.	1							2			Not Labeled	Not Labeled	Cat IV	Gautheron et al. (1994)
DL-Glutamic acid	19285-83-7	solid	20%	n.p.	2							1			Not Labeled			Gautheron et al. (1994)
DL-Glutamic acid	19285-83-7	solid	20%	n.p.	3							1			Not Labeled			Gautheron et al. (1994)
DL-Glutamic acid	19285-83-7	solid	20%	n.p.	4							1			Not Labeled			Gautheron et al. (1994)
DL-Glutamic acid	19285-83-7	solid	20%	n.p.	5							0			Not Labeled			Gautheron et al. (1994)
DL-Glutamic acid	19285-83-7	solid	20%	n.p.	6							-4			Not Labeled			Gautheron et al. (1994)
DL-Glutamic acid	19285-83-7	solid	20%	n.p.	7							1			Not Labeled			Gautheron et al. (1994)
DL-Glutamic acid	19285-83-7	solid	20%	n.p.	8							4			Mild			Gautheron et al. (1994)
DL-Glutamic acid	19285-83-7	solid	20%	n.p.	9							0			Not Labeled			Gautheron et al. (1994)
DL-Glutamic acid	19285-83-7	solid	20%	n.p.	10							2			Not Labeled			Gautheron et al. (1994)
DL-Glutamic acid	19285-83-7	solid	20%	n.p.	11							0			Not Labeled			Gautheron et al. (1994)
DL-Glutamic acid	19285-83-7	solid	20%	n.p.	12							-1			Not Labeled			Gautheron et al. (1994)
E		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	196.2	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
EDTA, di-potassium salt	25102-12-9	solid	20%	n.p.	1							-1			Not Labeled	Not Labeled	Cat IV	Gautheron et al. (1994)
EDTA, di-potassium salt	25102-12-9	solid	20%	n.p.	2							0			Not Labeled			Gautheron et al. (1994)
EDTA, di-potassium salt	25102-12-9	solid	20%	n.p.	3							-8			Not Labeled			Gautheron et al. (1994)
EDTA, di-potassium salt	25102-12-9	solid	20%	n.p.	4							2			Not Labeled			Gautheron et al. (1994)
EDTA, di-potassium salt	25102-12-9	solid	20%	n.p.	5							2			Not Labeled			Gautheron et al. (1994)
EDTA, di-potassium salt	25102-12-9	solid	20%	n.p.	6							2			Not Labeled			Gautheron et al. (1994)
EDTA, di-potassium salt	25102-12-9	solid	20%	n.p.	7							1			Not Labeled			Gautheron et al. (1994)
EDTA, di-potassium salt	25102-12-9	solid	20%	n.p.	8							-6			Not Labeled			Gautheron et al. (1994)
EDTA, di-potassium salt	25102-12-9	solid	20%	n.p.	9							1			Not Labeled			Gautheron et al. (1994)
EDTA, di-potassium salt	25102-12-9	solid	20%	n.p.	10							-1			Not Labeled			Gautheron et al. (1994)
EDTA, di-potassium salt	25102-12-9	solid	20%	n.p.	11							3			Not Labeled			Gautheron et al. (1994)
EDTA, di-potassium salt	25102-12-9	solid	20%	n.p.	12							1			Not Labeled			Gautheron et al. (1994)
EF		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	104.8	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
EG		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	71.8	n.p.	n.p.	Cat I	Cat I	Cat II	AMCP BRD
Ethanol	64-17-5	liquid	100%	n.p.	1	31			2.893			74.4			Severe	Severe	Cat I	Balls et al. (1995)
Ethanol	64-17-5	liquid	100%	n.p.	2	21.3			2.123			53.2			Moderate			Balls et al. (1995)
Ethanol	64-17-5	liquid	100%	n.p.	3	16.3			3.134			63.3			Severe			Balls et al. (1995)
Ethanol	64-17-5	liquid	100%	n.p.	4	36			4.134			98.01			Very severe			Balls et al. (1995)
Ethanol	64-17-5	liquid	100%	n.p.	5	30			2.277			64.2			Severe			Balls et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Ethanol	64-17-5	liquid	100%	n.p.	1							58			Severe	Severe	Cat I	Gautheron et al. (1994)
Ethanol	64-17-5	liquid	100%	n.p.	2							67			Severe			Gautheron et al. (1994)
Ethanol	64-17-5	liquid	100%	n.p.	3							70			Severe			Gautheron et al. (1994)
Ethanol	64-17-5	liquid	100%	n.p.	4							45			Moderate			Gautheron et al. (1994)
Ethanol	64-17-5	liquid	100%	n.p.	5							60			Severe			Gautheron et al. (1994)
Ethanol	64-17-5	liquid	100%	n.p.	6							64			Severe			Gautheron et al. (1994)
Ethanol	64-17-5	liquid	100%	n.p.	7							58			Severe			Gautheron et al. (1994)
Ethanol	64-17-5	liquid	100%	n.p.	8							51			Moderate			Gautheron et al. (1994)
Ethanol	64-17-5	liquid	100%	n.p.	9	22.3	6	4.1	1.56	6	0.316	46	6	6.6	Moderate			Gautheron et al. (1994)
Ethanol	64-17-5	liquid	100%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Ethanol	64-17-5	liquid	100%	n.p.	11							104			Severe			Gautheron et al. (1994)
Ethanol	64-17-5	liquid	100%	n.p.	12							45			Moderate			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Ethanol	64-17-5	liquid	100%	n.p.	1 (1)	17.6	3	2.3	1.265	3	0.252	36.6	3	6.0	Moderate	Moderate	Cat II	Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	1 (2)	16.4	3	5.5	1.415	3	0.389	37.6	3	10.8	Moderate			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	1 (3)	13.7	3	1.5	1.062	3	0.322	29.6	3	6.4	Moderate			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	1 (4)	12.7	3	1.0	1.933	3	0.397	41.7	3	5.8	Moderate			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	1 (5)	14.7	3	2.1	1.125	3	0.162	31.5	3	4.5	Moderate			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	1 (6)	12.7	3	14.9	1.995	3	0.035	42.6			Moderate			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	1 (7)	18.7	3	1.5	2.445	3	0.733	55.4			Severe			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	2 (1)	13.3	3	1.0	2.626	3	0.909	52.7	3	12.8	Moderate			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	2 (2)	17.0	3	2.3	2.504	3	0.703	54.5	3	8.3	Moderate			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	2 (3)	16.3	3	4.9	3.025	3	0.699	61.7	3	7.8	Severe			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	2 (4)	17.3	3	1.5	2.857	3	0.250	60.2	3	4.9	Severe			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	2 (5)	14.7	3	2.1	2.636	3	0.427	54.2	3	5.0	Moderate			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	2 (6)	17.6	3	0.6	3.718	3	0.798	73.4			Severe			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	2 (7)	15.0	3	2.6	3.267	3	0.545	64.0			Severe			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	2 (8)	13.0	3	0.6	2.561	3	0.867	51.4			Moderate			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	3 (1)	16.6	3	2.1	2.027	3	1.026	47.0	3	14.3	Moderate			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	3 (2)	18.0	3	2.9	1.831	3	0.061	45.4	3	2.0	Moderate			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	3 (3)	19.3	3	2.6	1.673	3	0.071	44.4	3	3.0	Moderate			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	3 (4)	22.0	3	2.6	1.583	3	0.426	45.7	3	8.5	Moderate			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	3 (5)	18.6	3	1.5	2.395	3	0.380	54.6	3	4.5	Moderate			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	3 (6)	17.0	3	1.2	1.853	3	0.268	44.8	3	5.1	Moderate			Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	3 (7)	19.3	3	3.8	1.527	3	0.344	42.2	3	8.8	Moderate			Southee (1998)
Ethyl acetate	141-78-6	liquid	100%	99	1	8.7			0.737			19.7			Mild	Moderate	Cat II	Balls et al. (1995)
Ethyl acetate	141-78-6	liquid	100%	99	2	5.7			1.513			28.4			Moderate			Balls et al. (1995)
Ethyl acetate	141-78-6	liquid	100%	99	3	9			2.543			47.1			Moderate			Balls et al. (1995)
Ethyl acetate	141-78-6	liquid	100%	99	4	13.33			2.065			44.31			Moderate			Balls et al. (1995)
Ethyl acetate	141-78-6	liquid	100%	99	5	11			0.64			20.6			Mild			Balls et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Ethyl acetoacetate	141-97-9	liquid	100%	n.p.	1							26			Moderate	Moderate	Cat II	Gautheron et al. (1994)
Ethyl acetoacetate	141-97-9	liquid	100%	n.p.	2							38			Moderate			Gautheron et al. (1994)
Ethyl acetoacetate	141-97-9	liquid	100%	n.p.	3							31			Moderate			Gautheron et al. (1994)
Ethyl acetoacetate	141-97-9	liquid	100%	n.p.	4							33			Moderate			Gautheron et al. (1994)
Ethyl acetoacetate	141-97-9	liquid	100%	n.p.	5							21			Moderate			Gautheron et al. (1994)
Ethyl acetoacetate	141-97-9	liquid	100%	n.p.	6							29			Moderate			Gautheron et al. (1994)
Ethyl acetoacetate	141-97-9	liquid	100%	n.p.	7							28			Moderate			Gautheron et al. (1994)
Ethyl acetoacetate	141-97-9	liquid	100%	n.p.	8							38			Moderate			Gautheron et al. (1994)
Ethyl acetoacetate	141-97-9	liquid	100%	n.p.	9							26			Moderate			Gautheron et al. (1994)
Ethyl acetoacetate	141-97-9	liquid	100%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Ethyl acetoacetate	141-97-9	liquid	100%	n.p.	11							38			Moderate			Gautheron et al. (1994)
Ethyl acetoacetate	141-97-9	liquid	100%	n.p.	12							42			Moderate			Gautheron et al. (1994)
Ethyl trimethyl acetate	3938-95-2	liquid	100%	99	1	10.3			1.136			27.4			Moderate	Mild	Cat III	Balls et al. (1995)
Ethyl trimethyl acetate	3938-95-2	liquid	100%	99	2	5			1.916			33.7			Moderate			Balls et al. (1995)
Ethyl trimethyl acetate	3938-95-2	liquid	100%	99	3	1.3			0.609			10.5			Mild			Balls et al. (1995)
Ethyl trimethyl acetate	3938-95-2	liquid	100%	99	4	5.33			0.22			8.633			Mild			Balls et al. (1995)
Ethyl trimethyl acetate	3938-95-2	liquid	100%	99	5	3.6			0.357			9			Mild			Balls et al. (1995)
Ethyl-2-methylacetoacetate	609-14-3	liquid	100%	97	1	26.7			0.052			27.5			Moderate	Mild	Cat III	Balls et al. (1995)
Ethyl-2-methylacetoacetate	609-14-3	liquid	100%	97	2	14.3			-0.014			14.1			Mild			Balls et al. (1995)
Ethyl-2-methylacetoacetate	609-14-3	liquid	100%	97	3	5.7			-0.012			5.5			Mild			Balls et al. (1995)
Ethyl-2-methylacetoacetate	609-14-3	liquid	100%	97	4	5.33			0.014			5.543			Mild			Balls et al. (1995)
Ethyl-2-methylacetoacetate	609-14-3	liquid	100%	97	5	18.7			0.061			19.6			Mild			Balls et al. (1995)
Ethylhexyl acid phosphate ester	-	liquid	100%	n.p.	-	117.3			0.880			130.5			Severe	Severe	Cat I	Bailey et al. (2004)
F		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	360.8	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
Floor Cleaner (#10)	-	liquid	100%	n.p.	-	45.2	5		1.675	5		70.3	5		Severe	Severe	Cat II	Swanson et al. (1995)
Floor Cleaner (#2)	-	liquid	100%	n.p.	-	-2.1	5		0.119	5		-0.3	5		Not Labeled	Not Labeled	Cat III	Swanson et al. (1995)
Floor Stripper (#14)	-	liquid	100%	n.p.	-	122.5	5		2.318	5		157.3	5		Severe	Severe	Cat I	Swanson et al. (1995)
Floor Stripper (#17)	-	liquid	100%	n.p.	-	180.5	5		2.38	5		216.2	5		Severe	Severe	Cat I	Swanson et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Floor Stripper (#18)	-	liquid	100%	n.p.	-	407.1	5		2.481	5		444.3	5		Severe	Severe	Cat I	Swanson et al. (1995)
Fomesafen	72128-02-0	solid	20%	97.5	2	4.3			9.837			151.9			Very severe	Severe	Cat II	Balls et al. (1995)
Fomesafen	72128-02-0	solid	20%	97.5	3	6.3			3.904			64.9			Severe			Balls et al. (1995)
Fomesafen	72128-02-0	solid	20%	97.5	4	13			0.668			23.023			Mild			Balls et al. (1995)
Fomesafen	72128-02-0	solid	20%	97.5	5	5.7			0.834			18.2			Mild			Balls et al. (1995)
Furan	110-00-9	liquid	100%	n.p.	1							73			Severe	Severe	Cat II	Gautheron et al. (1994)
Furan	110-00-9	liquid	100%	n.p.	2							63			Severe			Gautheron et al. (1994)
Furan	110-00-9	liquid	100%	n.p.	3							61			Severe			Gautheron et al. (1994)
Furan	110-00-9	liquid	100%	n.p.	4							65			Severe			Gautheron et al. (1994)
Furan	110-00-9	liquid	100%	n.p.	5							33			Moderate			Gautheron et al. (1994)
Furan	110-00-9	liquid	100%	n.p.	6							34			Moderate			Gautheron et al. (1994)
Furan	110-00-9	liquid	100%	n.p.	7							87			Severe			Gautheron et al. (1994)
Furan	110-00-9	liquid	100%	n.p.	8							48			Moderate			Gautheron et al. (1994)
Furan	110-00-9	liquid	100%	n.p.	9							50			Moderate			Gautheron et al. (1994)
Furan	110-00-9	liquid	100%	n.p.	10							39			Moderate			Gautheron et al. (1994)
Furan	110-00-9	liquid	100%	n.p.	11							68			Severe			Gautheron et al. (1994)
Furan	110-00-9	liquid	100%	n.p.	12							51			Moderate			Gautheron et al. (1994)
G		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	139.7	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
gamma-Butyrolactone	96-48-0	liquid	100%	>99	1	37.3			3.553			90.6			Very severe	Severe	Cat II	Balls et al. (1995)
gamma-Butyrolactone	96-48-0	liquid	100%	>99	2	22.7			0.682			32.9			Moderate			Balls et al. (1995)
gamma-Butyrolactone	96-48-0	liquid	100%	>99	3	22			0.63			31.5			Moderate			Balls et al. (1995)
gamma-Butyrolactone	96-48-0	liquid	100%	>99	4	48.67			2.192			81.55			Very severe			Balls et al. (1995)
gamma-Butyrolactone	96-48-0	liquid	100%	>99	5	31.7			2.357			67.1			Severe			Balls et al. (1995)
General Cleaner (#11)	-	liquid	100%	n.p.	-	77.9	5		0.359	5		83.3	5		Severe	Severe	Cat I	Swanson et al. (1995)
General Cleaner (#12)	-	liquid	100%	n.p.	-	95.5	5		1.197	5		113.5	5		Severe	Severe	Cat I	Swanson et al. (1995)
Glass Cleaner (#19)	-	liquid	100%	n.p.	-	98.3	5		2.499	5		135.8	5		Severe	Severe	Cat I	Swanson et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Gluconolactone	90-80-2	solid	20%	n.p.	1							63			Severe	Severe	Cat I	Gautheron et al. (1994)
Gluconolactone	90-80-2	solid	20%	n.p.	2							81			Severe			Gautheron et al. (1994)
Gluconolactone	90-80-2	solid	20%	n.p.	3							90			Severe			Gautheron et al. (1994)
Gluconolactone	90-80-2	solid	20%	n.p.	4							62			Severe			Gautheron et al. (1994)
Gluconolactone	90-80-2	solid	20%	n.p.	5							108			Severe			Gautheron et al. (1994)
Gluconolactone	90-80-2	solid	20%	n.p.	6							66			Severe			Gautheron et al. (1994)
Gluconolactone	90-80-2	solid	20%	n.p.	7							90			Severe			Gautheron et al. (1994)
Gluconolactone	90-80-2	solid	20%	n.p.	8							57			Severe			Gautheron et al. (1994)
Gluconolactone	90-80-2	solid	20%	n.p.	9							88			Severe			Gautheron et al. (1994)
Gluconolactone	90-80-2	solid	20%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Gluconolactone	90-80-2	solid	20%	n.p.	11							75			Severe			Gautheron et al. (1994)
Gluconolactone	90-80-2	solid	20%	n.p.	12							63			Severe			Gautheron et al. (1994)
Glycerol	56-81-5	liquid	100%	>99.5	1	-2			-0.001			-2			Not Labeled	Not Labeled	Cat IV	Balls et al. (1995)
Glycerol	56-81-5	liquid	100%	>99.5	2	-0.7			0.029			-0.2			Not Labeled			Balls et al. (1995)
Glycerol	56-81-5	liquid	100%	>99.5	3	0			0.018			0.3			Not Labeled			Balls et al. (1995)
Glycerol	56-81-5	liquid	100%	>99.5	4	3			0.005			3.08			Mild			Balls et al. (1995)
Glycerol	56-81-5	liquid	100%	>99.5	5	0			0.01			0.1			Not Labeled			Balls et al. (1995)
Glycerol	56-81-5	liquid	100%	>99.5	1 (1)	0.6	3	0.6	-0.005	3	0.002	0.6	3	0.6	Not Labeled	Not Labeled	Not Labeled	Southee (1998)
Glycerol	56-81-5	liquid	100%	>99.5	1 (2)	0.3	3	1.0	-0.003	3	0.002	0.3	3	1.0	Not Labeled			Southee (1998)
Glycerol	56-81-5	liquid	100%	>99.5	2 (1)	0.6	3	0.6	0.012	3	0.007	0.8	3	0.6	Not Labeled			Southee (1998)
Glycerol	56-81-5	liquid	100%	>99.5	2 (2)	0.7	3	0.6	0.008	3	0.009	0.8	3	0.7	Not Labeled			Southee (1998)
Glycerol	56-81-5	liquid	100%	>99.5	3 (1)	1.0	3	0.6	-0.003	3	0.005	1.0	3	0.6	Not Labeled			Southee (1998)
Glycerol	56-81-5	liquid	100%	>99.5	3 (2)	0.7	3	0.0	0.007	3	0.011	0.8	3	0.2	Not Labeled			Southee (1998)
H		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	14	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD
Heavy Duty Cleaner (#15)	-	liquid	100%	n.p.	-	323.3	5		2.24	5		357.1	5		Severe	Severe	Cat I	Swanson et al. (1995)
Heavy Duty Cleaner/Degreaser (#9)	-	liquid	100%	n.p.	-	315.4	5		2.619	5		354.7	5		Severe	Severe	Cat I	Swanson et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	1							93			Severe	Moderate	Cat II	Gautheron et al. (1994)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	2							40			Moderate			Gautheron et al. (1994)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	3							53			Moderate			Gautheron et al. (1994)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	4							33			Moderate			Gautheron et al. (1994)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	5							91			Severe			Gautheron et al. (1994)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	6							42			Moderate			Gautheron et al. (1994)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	7							82			Severe			Gautheron et al. (1994)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	8							76			Severe			Gautheron et al. (1994)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	9							70			Severe			Gautheron et al. (1994)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	11							48			Moderate			Gautheron et al. (1994)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	12							102			Severe			Gautheron et al. (1994)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	1 (1)	13.3	3	2.0	0.654	3	0.273	23.1	3	5.9	Mild	Moderate	Cat II	Southee (1998)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	1 (2)	9.7	3	4.2	0.499	3	0.109	17.2	3	5.8	Mild			Southee (1998)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	2 (1)	13.7	3	3.2	1.398	3	0.601	34.6	3	12.1	Moderate			Southee (1998)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	2 (2)	13.0	3	4.4	1.743	3	0.871	39.1	3	16.4	Moderate			Southee (1998)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	3 (1)	17.3	3	1.0	0.958	3	0.100	31.7	3	2.3	Moderate			Southee (1998)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	3 (2)	17.7	3	2.1	0.818	3	0.607	29.9	3	11.2	Moderate			Southee (1998)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Hexane	110-54-3	liquid	100%	n.p.	1							2			Not Labeled	Not Labeled	Cat IV	Gautheron et al. (1994)
Hexane	110-54-3	liquid	100%	n.p.	2							2			Not Labeled			Gautheron et al. (1994)
Hexane	110-54-3	liquid	100%	n.p.	3							2			Not Labeled			Gautheron et al. (1994)
Hexane	110-54-3	liquid	100%	n.p.	4							0			Not Labeled			Gautheron et al. (1994)
Hexane	110-54-3	liquid	100%	n.p.	5							2			Not Labeled			Gautheron et al. (1994)
Hexane	110-54-3	liquid	100%	n.p.	6							1			Not Labeled			Gautheron et al. (1994)
Hexane	110-54-3	liquid	100%	n.p.	7							3			Not Labeled			Gautheron et al. (1994)
Hexane	110-54-3	liquid	100%	n.p.	8							1			Not Labeled			Gautheron et al. (1994)
Hexane	110-54-3	liquid	100%	n.p.	9							1			Not Labeled			Gautheron et al. (1994)
Hexane	110-54-3	liquid	100%	n.p.	10							-1			Not Labeled			Gautheron et al. (1994)
Hexane	110-54-3	liquid	100%	n.p.	11							-2			Not Labeled			Gautheron et al. (1994)
Hexane	110-54-3	liquid	100%	n.p.	12							6			Mild			Gautheron et al. (1994)
I		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	0.6	n.p.	n.p.	Not Labeled	Not Labeled	Cat IV	AMCP BRD
Imidazole	288-32-4	solid	20%	99	1	68.3			3.232			116.8			Very severe	Severe	Cat I	Balls et al. (1995)
Imidazole	288-32-4	solid	20%	99	2	93			2.724			133.9			Very severe			Balls et al. (1995)
Imidazole	288-32-4	solid	20%	99	3	62.3			2.741			103.4			Very severe			Balls et al. (1995)
Imidazole	288-32-4	solid	20%	99	4	97.34			1.424			118.7			Very severe			Balls et al. (1995)
Imidazole	288-32-4	solid	20%	99	5	54.3			2.431			90.8			Very severe			Balls et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Imidazole	288-32-4	solid	20%	n.p.	1							75			Severe	Severe	Cat I	Gautheron et al. (1994)
Imidazole	288-32-4	solid	20%	n.p.	2							73			Severe			Gautheron et al. (1994)
Imidazole	288-32-4	solid	20%	n.p.	3							140			Severe			Gautheron et al. (1994)
Imidazole	288-32-4	solid	20%	n.p.	4							81			Severe			Gautheron et al. (1994)
Imidazole	288-32-4	solid	20%	n.p.	5							96			Severe			Gautheron et al. (1994)
Imidazole	288-32-4	solid	20%	n.p.	6							62			Severe			Gautheron et al. (1994)
Imidazole	288-32-4	solid	20%	n.p.	7							82			Severe			Gautheron et al. (1994)
Imidazole	288-32-4	solid	20%	n.p.	8							122			Severe			Gautheron et al. (1994)
Imidazole	288-32-4	solid	20%	n.p.	9							64			Severe			Gautheron et al. (1994)
Imidazole	288-32-4	solid	20%	n.p.	10							81			Severe			Gautheron et al. (1994)
Imidazole	288-32-4	solid	20%	n.p.	11							114			Severe			Gautheron et al. (1994)
Imidazole	288-32-4	solid	20%	n.p.	12							65			Severe			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Imidazole	288-32-4	solid	20%	n.p.	1 (1)	91.3	3	2.1	3.379	3	0.106	142.0	3	3.0	Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	1 (2)	88.0	3	7.5	3.306	3	0.597	137.6	3	6.8	Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	1 (3)	73.7	3	10.1	2.565	3	1.063	112.2	3	24.7	Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	1 (4)	86.0	3	9.6	3.006	3	1.078	131.1	3	6.7	Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	1 (5)	97.0	3	15.5	3.241	3	0.233	145.6	3	12.0	Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	1 (6)	115.3	3	9.1	3.150	3	0.181	162.6	3		Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	1 (7)	70.3	3	4.5	3.681	3	0.691	125.5	3		Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	2 (1)	85.7	3	9.8	3.490	3	0.309	138.1	3	13.0	Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	2 (2)	88.0	3	13.0	3.471	3	0.381	140.1	3	11.9	Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	2 (3)	86.3	3	6.0	3.240	3	0.651	134.9	3	9.4	Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	2 (4)	92.3	3	7.9	4.324	3	1.048	157.2	3	12.5	Very severe	Very Severe	Very Severe	Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	2 (5)	88.0	3	16.7	3.308	3	0.695	137.6	3	6.8	Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	2 (6)	97.3	3	12.9	3.709	3	0.866	152.9			Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	2 (7)	100.0	3	9.1	3.316	3	0.183	148.7			Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	3 (1)	83.0	3	14.8	3.774	3	0.828	139.6	3	26.0	Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	3 (2)	91.7	3	9.3	3.232	3	0.702	140.1	3	18.9	Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	3 (3)	80.4	3	3.1	2.907	3	0.642	124.0	3	6.9	Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	3 (4)	82.3	3	2.1	3.093	3	0.635	128.7	3	8.2	Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	3 (5)	76.6	3	8.3	3.118	3	0.464	123.4	3	14.8	Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	3 (6)	76.3	3	8.7	2.862	3	0.292	121.2	3	4.6	Very severe			Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	3 (7)	77.3	3	2.0	3.602	3	0.413	131.3	3	8.2	Very severe			Southee (1998)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Iminodibenzyl	494-19-9	solid	20%	n.p.	1							0			Not Labeled	Not Labeled	Cat IV	Gautheron et al. (1994)
Iminodibenzyl	494-19-9	solid	20%	n.p.	2							1			Not Labeled			Gautheron et al. (1994)
Iminodibenzyl	494-19-9	solid	20%	n.p.	3							6			Mild			Gautheron et al. (1994)
Iminodibenzyl	494-19-9	solid	20%	n.p.	4							0			Not Labeled			Gautheron et al. (1994)
Iminodibenzyl	494-19-9	solid	20%	n.p.	5							4			Mild			Gautheron et al. (1994)
Iminodibenzyl	494-19-9	solid	20%	n.p.	6							0			Not Labeled			Gautheron et al. (1994)
Iminodibenzyl	494-19-9	solid	20%	n.p.	7							1			Not Labeled			Gautheron et al. (1994)
Iminodibenzyl	494-19-9	solid	20%	n.p.	8							12			Mild			Gautheron et al. (1994)
Iminodibenzyl	494-19-9	solid	20%	n.p.	9							0			Not Labeled			Gautheron et al. (1994)
Iminodibenzyl	494-19-9	solid	20%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Iminodibenzyl	494-19-9	solid	20%	n.p.	11							6			Mild			Gautheron et al. (1994)
Iminodibenzyl	494-19-9	solid	20%	n.p.	12							-4			Not Labeled			Gautheron et al. (1994)
Isobutanol	78-83-1	liquid	100%	99.9	1	17			2.494			54.4			Moderate	Moderate	Cat II	Balls et al. (1995)
Isobutanol	78-83-1	liquid	100%	99.9	2	20			3.598			74			Severe			Balls et al. (1995)
Isobutanol	78-83-1	liquid	100%	99.9	3	19			3.248			67.7			Severe			Balls et al. (1995)
Isobutanol	78-83-1	liquid	100%	99.9	4	26			1.052			41.78			Moderate			Balls et al. (1995)
Isobutanol	78-83-1	liquid	100%	99.9	5	21.4			1.39			42.2			Moderate			Balls et al. (1995)
Isopropanol	67-63-0	liquid	100%	99.9	1	11.7			1.868			39.7			Moderate	Severe	Cat II	Balls et al. (1995)
Isopropanol	67-63-0	liquid	100%	99.9	2	23.3			2.409			59.5			Severe			Balls et al. (1995)
Isopropanol	67-63-0	liquid	100%	99.9	3	16			3.755			72.3			Severe			Balls et al. (1995)
Isopropanol	67-63-0	liquid	100%	99.9	4	30.66			3.189			78.5			Severe			Balls et al. (1995)
Isopropanol	67-63-0	liquid	100%	99.9	5	18.3			1.4			39.3			Moderate			Balls et al. (1995)
J		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	7.7	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD
K		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	0.3	n.p.	n.p.	Not Labeled	Not Labeled	Cat IV	AMCP BRD
L		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	5.5	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
L-Aspartic acid	70-47-3	solid	20%	100	1	2			-0.011			1.8			Not Labeled	Not Labeled	Cat IV	Balls et al. (1995)
L-Aspartic acid	70-47-3	solid	20%	100	2	1.7			-0.107			0.1			Not Labeled			Balls et al. (1995)
L-Aspartic acid	70-47-3	solid	20%	100	3	2.7			-0.003			2.6			Not Labeled			Balls et al. (1995)
L-Aspartic acid	70-47-3	solid	20%	100	4	0.33			0.03			0.788			Not Labeled			Balls et al. (1995)
L-Aspartic acid	70-47-3	solid	20%	100	5	0			0.082			1.2			Not Labeled			Balls et al. (1995)
Laurylsulfobetaine	14933-08-5	surfactant	10%	n.p.	1							81			Severe	Severe	Cat I	Gautheron et al. (1994)
Laurylsulfobetaine	14933-08-5	surfactant	10%	n.p.	2							82			Severe			Gautheron et al. (1994)
Laurylsulfobetaine	14933-08-5	surfactant	10%	n.p.	3							103			Severe			Gautheron et al. (1994)
Laurylsulfobetaine	14933-08-5	surfactant	10%	n.p.	4							76			Severe			Gautheron et al. (1994)
Laurylsulfobetaine	14933-08-5	surfactant	10%	n.p.	5							92			Severe			Gautheron et al. (1994)
Laurylsulfobetaine	14933-08-5	surfactant	10%	n.p.	6							68			Severe			Gautheron et al. (1994)
Laurylsulfobetaine	14933-08-5	surfactant	10%	n.p.	7							90			Severe			Gautheron et al. (1994)
Laurylsulfobetaine	14933-08-5	surfactant	10%	n.p.	8							62			Severe			Gautheron et al. (1994)
Laurylsulfobetaine	14933-08-5	surfactant	10%	n.p.	9							102			Severe			Gautheron et al. (1994)
Laurylsulfobetaine	14933-08-5	surfactant	10%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Laurylsulfobetaine	14933-08-5	surfactant	10%	n.p.	11							76			Severe			Gautheron et al. (1994)
Laurylsulfobetaine	14933-08-5	surfactant	10%	n.p.	12							55			Moderate			Gautheron et al. (1994)
M		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	55.7	n.p.	n.p.	Cat I	Cat I	Cat II	AMCP BRD

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Magnesium carbonate	56378-72-4	solid	20%	n.p.	1							3			Not Labeled	Not Labeled	Cat IV	Gautheron et al. (1994)
Magnesium carbonate	56378-72-4	solid	20%	n.p.	2							6			Mild			Gautheron et al. (1994)
Magnesium carbonate	56378-72-4	solid	20%	n.p.	3							3			Not Labeled			Gautheron et al. (1994)
Magnesium carbonate	56378-72-4	solid	20%	n.p.	4							3			Not Labeled			Gautheron et al. (1994)
Magnesium carbonate	56378-72-4	solid	20%	n.p.	5							0			Not Labeled			Gautheron et al. (1994)
Magnesium carbonate	56378-72-4	solid	20%	n.p.	6							1			Not Labeled			Gautheron et al. (1994)
Magnesium carbonate	56378-72-4	solid	20%	n.p.	7							7			Mild			Gautheron et al. (1994)
Magnesium carbonate	56378-72-4	solid	20%	n.p.	8							3			Not Labeled			Gautheron et al. (1994)
Magnesium carbonate	56378-72-4	solid	20%	n.p.	9							1			Not Labeled			Gautheron et al. (1994)
Magnesium carbonate	56378-72-4	solid	20%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Magnesium carbonate	56378-72-4	solid	20%	n.p.	11							0			Not Labeled			Gautheron et al. (1994)
Magnesium carbonate	56378-72-4	solid	20%	n.p.	12							6			Mild			Gautheron et al. (1994)
Maneb	12427-38-2	solid	20%	90	2	17			-0.008			16.9			Mild	Mild	Cat II	Balls et al. (1995)
Maneb	12427-38-2	solid	20%	90	3	21			-0.002			21			Mild			Balls et al. (1995)
Maneb	12427-38-2	solid	20%	90	4	56.33			0.495			63.76			Severe			Balls et al. (1995)
Maneb	12427-38-2	solid	20%	90	5	33.3			0.029			33.8			Moderate			Balls et al. (1995)
Meat Room Degreaser (#3)	-	liquid	100%	n.p.	-	99.3	5		2.733	5		140.3	5		Severe	Severe	Cat I	Swanson et al. (1995)
Metal Cleaner (#20)	-	liquid	100%	n.p.	-	344.2	5		3.182	5		391.9	5		Severe	Severe	Cat I	Swanson et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Methanol	67-56-1	liquid	100%	n.p.	1							88			Severe	Severe	Cat I	Gautheron et al. (1994)
Methanol	67-56-1	liquid	100%	n.p.	2							88			Severe			Gautheron et al. (1994)
Methanol	67-56-1	liquid	100%	n.p.	3							54			Moderate			Gautheron et al. (1994)
Methanol	67-56-1	liquid	100%	n.p.	4							71			Severe			Gautheron et al. (1994)
Methanol	67-56-1	liquid	100%	n.p.	5							81			Severe			Gautheron et al. (1994)
Methanol	67-56-1	liquid	100%	n.p.	6							108			Severe			Gautheron et al. (1994)
Methanol	67-56-1	liquid	100%	n.p.	7							37			Moderate			Gautheron et al. (1994)
Methanol	67-56-1	liquid	100%	n.p.	8							19			Mild			Gautheron et al. (1994)
Methanol	67-56-1	liquid	100%	n.p.	9							99			Severe			Gautheron et al. (1994)
Methanol	67-56-1	liquid	100%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Methanol	67-56-1	liquid	100%	n.p.	11							179			Severe			Gautheron et al. (1994)
Methanol	67-56-1	liquid	100%	n.p.	12							102			Severe			Gautheron et al. (1994)
Methyl acetate	79-20-9	liquid	100%	98	1	51.6			1.301			71.2			Severe	Moderate	Cat II	Balls et al. (1995)
Methyl acetate	79-20-9	liquid	100%	98	2	42			0.299			46.5			Moderate			Balls et al. (1995)
Methyl acetate	79-20-9	liquid	100%	98	3	38.3			0.887			51.6			Moderate			Balls et al. (1995)
Methyl acetate	79-20-9	liquid	100%	98	4	43.1			0.72			53.9			Moderate			Balls et al. (1995)
Methyl acetate	79-20-9	liquid	100%	98	5	45.3			0.384			51.1			Moderate			Balls et al. (1995)
Methyl cyanoacetate	105-34-0	liquid	100%	99	1	16.3			0.002			16.3			Mild	Mild	Cat III	Balls et al. (1995)
Methyl cyanoacetate	105-34-0	liquid	100%	99	2	6.7			-0.052			5.9			Mild			Balls et al. (1995)
Methyl cyanoacetate	105-34-0	liquid	100%	99	3	10.3			-0.015			10.1			Mild			Balls et al. (1995)
Methyl cyanoacetate	105-34-0	liquid	100%	99	4	17.33			0.013			17.53			Mild			Balls et al. (1995)
Methyl cyanoacetate	105-34-0	liquid	100%	99	5	11			-0.003			11			Mild			Balls et al. (1995)
Methyl cyclopentadiene dimer	-	liquid	100%	n.p.	-	0.7			0.001			0.7			Not Labeled	Not Labeled	Cat IV	Bailey et al. (2004)
Methyl ethyl ketone	78-93-3	liquid	100%	99	1	68			1.665			93			Very severe	Severe	Cat II	Balls et al. (1995)
Methyl ethyl ketone	78-93-3	liquid	100%	99	2	51.3			1.069			67.4			Severe			Balls et al. (1995)
Methyl ethyl ketone	78-93-3	liquid	100%	99	3	34			1.212			52.2			Moderate			Balls et al. (1995)
Methyl ethyl ketone	78-93-3	liquid	100%	99	4	58			1.38			78.71			Severe			Balls et al. (1995)
Methyl ethyl ketone	78-93-3	liquid	100%	99	5	51.7			0.607			60.8			Severe			Balls et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Methyl ethyl ketone	78-93-3	liquid	100%	99	1 (1)	47.6	3	5.9	1.706	3	0.679	73.3	3	15.9	Severe	Severe	Cat II	Southee (1998)
Methyl ethyl ketone	78-93-3	liquid	100%	99	1 (2)	48	3	2.1	1.32	3	0.303	67.8	3	5.7	Severe			Southee (1998)
Methyl ethyl ketone	78-93-3	liquid	100%	99	2 (1)	61	3	2.9	3.183	3	0.86	108.7	3	11.9	Very severe			Southee (1998)
Methyl ethyl ketone	78-93-3	liquid	100%	99	2 (2)	62	3	6.7	2.648	3	1.074	101.7	3	21.1	Very severe			Southee (1998)
Methyl ethyl ketone	78-93-3	liquid	100%	99	3 (1)	55.7	3	5.0	0.972	3	0.479	70.2	3	3.5	Severe			Southee (1998)
Methyl ethyl ketone	78-93-3	liquid	100%	99	3 (2)	54.4	3	1.5	1.278	3	0.359	73.5	3	6.4	Severe			Southee (1998)
Methyl isobutyl ketone	108-10-1	liquid	100%	98	1	4.7			0.273			8.8			Mild	Mild	Cat III	Balls et al. (1995)
Methyl isobutyl ketone	108-10-1	liquid	100%	98	2	8.7			0.759			20.1			Mild			Balls et al. (1995)
Methyl isobutyl ketone	108-10-1	liquid	100%	98	3	5.7			0.307			10.3			Mild			Balls et al. (1995)
Methyl isobutyl ketone	108-10-1	liquid	100%	98	4	8			0.35			13.25			Mild			Balls et al. (1995)
Methyl isobutyl ketone	108-10-1	liquid	100%	98	5	5.7			0.305			10.3			Mild			Balls et al. (1995)
Methylcyclopentane	96-37-7	liquid	100%	>99	1	1.3			0.169			3.8			Mild	Not Labeled	Cat IV	Balls et al. (1995)
Methylcyclopentane	96-37-7	liquid	100%	>99	2	2.3			0.152			4.6			Mild			Balls et al. (1995)
Methylcyclopentane	96-37-7	liquid	100%	>99	3	0.3			0.071			1.4			Not Labeled			Balls et al. (1995)
Methylcyclopentane	96-37-7	liquid	100%	>99	4	1			0.047			1.71			Not Labeled			Balls et al. (1995)
Methylcyclopentane	96-37-7	liquid	100%	>99	5	0.3			0.161			2.7			Not Labeled			Balls et al. (1995)
Methylisobutyl ketone	108-10-1	liquid	100%	n.p.	1							22			Mild	Mild		Gautheron et al. (1994)
Methylisobutyl ketone	108-10-1	liquid	100%	n.p.	2							25			Mild			Gautheron et al. (1994)
Methylisobutyl ketone	108-10-1	liquid	100%	n.p.	3							27			Moderate			Gautheron et al. (1994)
Methylisobutyl ketone	108-10-1	liquid	100%	n.p.	4							19			Mild			Gautheron et al. (1994)
Methylisobutyl ketone	108-10-1	liquid	100%	n.p.	5							21			Mild			Gautheron et al. (1994)
Methylisobutyl ketone	108-10-1	liquid	100%	n.p.	6							23			Mild			Gautheron et al. (1994)
Methylisobutyl ketone	108-10-1	liquid	100%	n.p.	7							16			Mild			Gautheron et al. (1994)
Methylisobutyl ketone	108-10-1	liquid	100%	n.p.	8							16			Mild			Gautheron et al. (1994)
Methylisobutyl ketone	108-10-1	liquid	100%	n.p.	9							19			Mild			Gautheron et al. (1994)
Methylisobutyl ketone	108-10-1	liquid	100%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Methylisobutyl ketone	108-10-1	liquid	100%	n.p.	11							20			Mild			Gautheron et al. (1994)
Methylisobutyl ketone	108-10-1	liquid	100%	n.p.	12							11			Mild			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
MYRJ-45	-	surfactant	10%	n.p.	1							2			Not Labeled	Not Labeled	Cat IV	Gautheron et al. (1994)
MYRJ-45	-	surfactant	10%	n.p.	2							1			Not Labeled			Gautheron et al. (1994)
MYRJ-45	-	surfactant	10%	n.p.	3							0			Not Labeled			Gautheron et al. (1994)
MYRJ-45	-	surfactant	10%	n.p.	4							1			Not Labeled			Gautheron et al. (1994)
MYRJ-45	-	surfactant	10%	n.p.	5							0			Not Labeled			Gautheron et al. (1994)
MYRJ-45	-	surfactant	10%	n.p.	6							0			Not Labeled			Gautheron et al. (1994)
MYRJ-45	-	surfactant	10%	n.p.	7							1			Not Labeled			Gautheron et al. (1994)
MYRJ-45	-	surfactant	10%	n.p.	8							-4			Not Labeled			Gautheron et al. (1994)
MYRJ-45	-	surfactant	10%	n.p.	9							1			Not Labeled			Gautheron et al. (1994)
MYRJ-45	-	surfactant	10%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
MYRJ-45	-	surfactant	10%	n.p.	11							-3			Not Labeled			Gautheron et al. (1994)
MYRJ-45	-	surfactant	10%	n.p.	12							-1			Not Labeled			Gautheron et al. (1994)
N		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	152.7	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
n-Hexanol	111-27-3	liquid	100%	98	1	17.7			3.591			71.5			Severe	Severe/Very Severe	Cat II	Balls et al. (1995)
n-Hexanol	111-27-3	liquid	100%	98	2	16			4.509			83.6			Very severe			Balls et al. (1995)
n-Hexanol	111-27-3	liquid	100%	98	3	7			3.746			63.2			Severe			Balls et al. (1995)
n-Hexanol	111-27-3	liquid	100%	98	4	15.33			2.191			48.19			Moderate			Balls et al. (1995)
n-Hexanol	111-27-3	liquid	100%	98	5	10.7			2.145			42.9			Moderate			Balls et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
N-Lauroylsarcosine, sodium salt	7631-98-3	surfactant	10%	n.p.	1							53			Moderate	Moderate	Cat II	Gautheron et al. (1994)
N-Lauroylsarcosine, sodium salt	7631-98-3	surfactant	10%	n.p.	2							50			Moderate			Gautheron et al. (1994)
N-Lauroylsarcosine, sodium salt	7631-98-3	surfactant	10%	n.p.	3							48			Moderate			Gautheron et al. (1994)
N-Lauroylsarcosine, sodium salt	7631-98-3	surfactant	10%	n.p.	4							28			Moderate			Gautheron et al. (1994)
N-Lauroylsarcosine, sodium salt	7631-98-3	surfactant	10%	n.p.	5							45			Moderate			Gautheron et al. (1994)
N-Lauroylsarcosine, sodium salt	7631-98-3	surfactant	10%	n.p.	6							35			Moderate			Gautheron et al. (1994)
N-Lauroylsarcosine, sodium salt	7631-98-3	surfactant	10%	n.p.	7							48			Moderate			Gautheron et al. (1994)
N-Lauroylsarcosine, sodium salt	7631-98-3	surfactant	10%	n.p.	8							43			Moderate			Gautheron et al. (1994)
N-Lauroylsarcosine, sodium salt	7631-98-3	surfactant	10%	n.p.	9							63			Severe			Gautheron et al. (1994)
N-Lauroylsarcosine, sodium salt	7631-98-3	surfactant	10%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
N-Lauroylsarcosine, sodium salt	7631-98-3	surfactant	10%	n.p.	11							89			Severe			Gautheron et al. (1994)
N-Lauroylsarcosine, sodium salt	7631-98-3	surfactant	10%	n.p.	12							48			Moderate			Gautheron et al. (1994)
n-Octanol	111-87-5	liquid	100%	>99	1	11			2.159			43.4			Moderate	Moderate	Cat II	Balls et al. (1995)
n-Octanol	111-87-5	liquid	100%	>99	2	13			4.392			78.9			Severe			Balls et al. (1995)
n-Octanol	111-87-5	liquid	100%	>99	3	10			1.984			39.8			Moderate			Balls et al. (1995)
n-Octanol	111-87-5	liquid	100%	>99	4	6			0.569			14.54			Mild			Balls et al. (1995)
n-Octanol	111-87-5	liquid	100%	>99	5	6			1.464			28			Moderate			Balls et al. (1995)
O		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	7.2	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Octanol	111-87-5	liquid	100%	n.p.	1							65			Severe	Moderate	Cat II	Gautheron et al. (1994)
Octanol	111-87-5	liquid	100%	n.p.	2							33			Moderate			Gautheron et al. (1994)
Octanol	111-87-5	liquid	100%	n.p.	3							42			Moderate			Gautheron et al. (1994)
Octanol	111-87-5	liquid	100%	n.p.	4							49			Moderate			Gautheron et al. (1994)
Octanol	111-87-5	liquid	100%	n.p.	5							66			Severe			Gautheron et al. (1994)
Octanol	111-87-5	liquid	100%	n.p.	6							48			Moderate			Gautheron et al. (1994)
Octanol	111-87-5	liquid	100%	n.p.	7							37			Moderate			Gautheron et al. (1994)
Octanol	111-87-5	liquid	100%	n.p.	8							25			Mild			Gautheron et al. (1994)
Octanol	111-87-5	liquid	100%	n.p.	9							61			Severe			Gautheron et al. (1994)
Octanol	111-87-5	liquid	100%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Octanol	111-87-5	liquid	100%	n.p.	11							31			Moderate			Gautheron et al. (1994)
Octanol	111-87-5	liquid	100%	n.p.	12							64			Severe			Gautheron et al. (1994)
P		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	1.1	n.p.	n.p.	Not Labeled	Not Labeled	Cat IV	AMCP BRD
Parafluoraniline	371-40-4	liquid	100%	99	1	17.3			0.809			29.5			Moderate	Moderate	Cat II	Balls et al. (1995)
Parafluoraniline	371-40-4	liquid	100%	99	2	11.3			1.006			26.4			Moderate			Balls et al. (1995)
Parafluoraniline	371-40-4	liquid	100%	99	3	18.7			1.474			40.8			Moderate			Balls et al. (1995)
Parafluoraniline	371-40-4	liquid	100%	99	4	18			0.8996			31.82			Moderate			Balls et al. (1995)
Parafluoraniline	371-40-4	liquid	100%	99	5	13.3			0.679			23.5			Moderate			Balls et al. (1995)
Parafluoraniline	371-40-4	liquid	100%	99	1 (1)	15.3	3	1.0	1.044	3	0.413	31	3	7.2	Moderate	Moderate	Cat II	Southee (1998)
Parafluoraniline	371-40-4	liquid	100%	99	1 (2)	16.3	3	3.5	1.243	3	0.287	35	3	6.2	Moderate			Southee (1998)
Parafluoraniline	371-40-4	liquid	100%	99	2 (1)	13.3	3	2.1	1.663	3	0.372	38.3	3	7.5	Moderate			Southee (1998)
Parafluoraniline	371-40-4	liquid	100%	99	2 (2)	16.0	3	4.6	1.432	3	0.531	37.5	3	12.2	Moderate			Southee (1998)
Parafluoraniline	371-40-4	liquid	100%	99	3 (1)	11.0	3	1.0	0.738	3	0.154	22.1	3	2.7	Mild			Southee (1998)
Parafluoraniline	371-40-4	liquid	100%	99	3 (2)	15.4	3	1.2	0.7	3	0.151	28.9	3	3.4	Moderate			Southee (1998)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Petroleum ether	8032-32-4	liquid	100%	n.p.	1							8			Mild	Mild	Cat IV	Gautheron et al. (1994)
Petroleum ether	8032-32-4	liquid	100%	n.p.	2							13			Mild			Gautheron et al. (1994)
Petroleum ether	8032-32-4	liquid	100%	n.p.	3							11			Mild			Gautheron et al. (1994)
Petroleum ether	8032-32-4	liquid	100%	n.p.	4							1			Not Labeled			Gautheron et al. (1994)
Petroleum ether	8032-32-4	liquid	100%	n.p.	5							2			Not Labeled			Gautheron et al. (1994)
Petroleum ether	8032-32-4	liquid	100%	n.p.	6							5			Mild			Gautheron et al. (1994)
Petroleum ether	8032-32-4	liquid	100%	n.p.	7							7			Mild			Gautheron et al. (1994)
Petroleum ether	8032-32-4	liquid	100%	n.p.	8							0			Not Labeled			Gautheron et al. (1994)
Petroleum ether	8032-32-4	liquid	100%	n.p.	9							2			Not Labeled			Gautheron et al. (1994)
Petroleum ether	8032-32-4	liquid	100%	n.p.	10							3			Not Labeled			Gautheron et al. (1994)
Petroleum ether	8032-32-4	liquid	100%	n.p.	11							5			Mild			Gautheron et al. (1994)
Petroleum ether	8032-32-4	liquid	100%	n.p.	12							9			Mild			Gautheron et al. (1994)
Petroleum wax	-	solid	100%	n.p.	-	0.3			-0.001			0.3			Not Labeled	Not Labeled	Cat IV	Bailey et al. (2004)
Phenylbutazone	50-33-9	solid	20%	n.p.	1							0			Not Labeled	Not Labeled	Cat IV	Gautheron et al. (1994)
Phenylbutazone	50-33-9	solid	20%	n.p.	2							1			Not Labeled			Gautheron et al. (1994)
Phenylbutazone	50-33-9	solid	20%	n.p.	3							1			Not Labeled			Gautheron et al. (1994)
Phenylbutazone	50-33-9	solid	20%	n.p.	4							0			Not Labeled			Gautheron et al. (1994)
Phenylbutazone	50-33-9	solid	20%	n.p.	5							0			Not Labeled			Gautheron et al. (1994)
Phenylbutazone	50-33-9	solid	20%	n.p.	6							1			Not Labeled			Gautheron et al. (1994)
Phenylbutazone	50-33-9	solid	20%	n.p.	7							0			Not Labeled			Gautheron et al. (1994)
Phenylbutazone	50-33-9	solid	20%	n.p.	8							-6			Not Labeled			Gautheron et al. (1994)
Phenylbutazone	50-33-9	solid	20%	n.p.	9							1			Not Labeled			Gautheron et al. (1994)
Phenylbutazone	50-33-9	solid	20%	n.p.	10							1			Not Labeled			Gautheron et al. (1994)
Phenylbutazone	50-33-9	solid	20%	n.p.	11							-3			Not Labeled			Gautheron et al. (1994)
Phenylbutazone	50-33-9	solid	20%	n.p.	12							2			Not Labeled			Gautheron et al. (1994)
Polyalkenylsuccinate ester/amine salt	-	liquid	100%	n.p.	-	2.3			0.000			2.3			Not Labeled	Not Labeled	Cat IV	Bailey et al. (2004)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Polyethylene glycol 400	25322-68-3	liquid	100%	n.p.	1	0.3			0.019			0.6			Not Labeled	Not Labeled	Cat IV	Balls et al. (1995)
Polyethylene glycol 400	25322-68-3	liquid	100%	n.p.	2	2			0.036			2.5			Not Labeled			Balls et al. (1995)
Polyethylene glycol 400	25322-68-3	liquid	100%	n.p.	3	-1.7			0.021			-1.3			Not Labeled			Balls et al. (1995)
Polyethylene glycol 400	25322-68-3	liquid	100%	n.p.	4	1			0.005			1.08			Not Labeled			Balls et al. (1995)
Polyethylene glycol 400	25322-68-3	liquid	100%	n.p.	5	2.7			0.01			2.8			Not Labeled			Balls et al. (1995)
Pot and Pan Cleaner (#8)	-	liquid	100%	n.p.	-	-1.8	5		0.078	5		-0.6	5		Not Labeled	Not Labeled	Cat IV	Swanson et al. (1995)
Potassium cyanate	590-28-3	solid	20%	97	1	8.7			0.499			16.2			Mild	Mild	Cat III	Balls et al. (1995)
Potassium cyanate	590-28-3	solid	20%	97	2	11			0.793			22.9			Mild			Balls et al. (1995)
Potassium cyanate	590-28-3	solid	20%	97	3	8.3			0.248			12			Mild			Balls et al. (1995)
Potassium cyanate	590-28-3	solid	20%	97	4	7			0.692			17.38			Mild			Balls et al. (1995)
Potassium cyanate	590-28-3	solid	20%	97	5	3			0.234			6.5			Mild			Balls et al. (1995)
Process oil	-	liquid	100%	n.p.	-	2.7			0.004			2.7			Not Labeled	Not Labeled	Cat IV	Bailey et al. (2004)
Promethazine hydrochloride	58-33-3	solid	20%	98	1	120.7			-0.022			120.3			Very severe	Severe	Cat I	Balls et al. (1995)
Promethazine hydrochloride	58-33-3	solid	20%	98	2	87.7			-0.234			84.2			Very severe			Balls et al. (1995)
Promethazine hydrochloride	58-33-3	solid	20%	98	3	125			0.044			125.7			Very severe			Balls et al. (1995)
Promethazine hydrochloride	58-33-3	solid	20%	98	4	121.33			0.051			123.09			Very severe			Balls et al. (1995)
Promethazine hydrochloride	58-33-3	solid	20%	98	5	153.7			0.011			153.8			Very severe			Balls et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Promethazine hydrochloride	58-33-3	solid	20%	n.p.	1							117			Severe	Severe	Cat I	Gautheron et al. (1994)
Promethazine hydrochloride	58-33-3	solid	20%	n.p.	2							156			Severe			Gautheron et al. (1994)
Promethazine hydrochloride	58-33-3	solid	20%	n.p.	3							109			Severe			Gautheron et al. (1994)
Promethazine hydrochloride	58-33-3	solid	20%	n.p.	4							111			Severe			Gautheron et al. (1994)
Promethazine hydrochloride	58-33-3	solid	20%	n.p.	5							164			Severe			Gautheron et al. (1994)
Promethazine hydrochloride	58-33-3	solid	20%	n.p.	6							174			Severe			Gautheron et al. (1994)
Promethazine hydrochloride	58-33-3	solid	20%	n.p.	7							103			Severe			Gautheron et al. (1994)
Promethazine hydrochloride	58-33-3	solid	20%	n.p.	8							50			Moderate			Gautheron et al. (1994)
Promethazine hydrochloride	58-33-3	solid	20%	n.p.	9							139			Severe			Gautheron et al. (1994)
Promethazine hydrochloride	58-33-3	solid	20%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Promethazine hydrochloride	58-33-3	solid	20%	n.p.	11							94			Severe			Gautheron et al. (1994)
Promethazine hydrochloride	58-33-3	solid	20%	n.p.	12							19			Mild			Gautheron et al. (1994)
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	n.p.	1							7			Mild	Mild	Cat III	Gautheron et al. (1994)
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	n.p.	2							7			Mild			Gautheron et al. (1994)
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	n.p.	3							14			Mild			Gautheron et al. (1994)
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	n.p.	4							4			Mild			Gautheron et al. (1994)
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	n.p.	5							6			Mild			Gautheron et al. (1994)
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	n.p.	6							9			Mild			Gautheron et al. (1994)
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	n.p.	7							6			Mild			Gautheron et al. (1994)
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	n.p.	8							11			Mild			Gautheron et al. (1994)
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	n.p.	9							6			Mild			Gautheron et al. (1994)
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	n.p.	11							12			Mild			Gautheron et al. (1994)
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	n.p.	12							5			Mild			Gautheron et al. (1994)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	100	1 (1)	10.7	3	2.6	0.034	3	0.044	11.2	3	3.2	Mild	Mild	Cat III	Southee (1998)
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	100	1 (2)	7.0	3	0.6	0.023	3	0.026	7.4	3	0.6	Mild			
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	100	2 (1)	5.0	3	1.7	0.013	3	0.012	5.2	3	1.9	Mild			
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	100	2 (2)	3.4	3	1.5	0.016	3	0.015	3.6	3	1.6	Mild			
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	100	3 (1)	7.3	3	4.4	0.028	3	0.014	7.7	3	4.2	Mild			
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	100	3 (2)	5.6	3	0.6	0.04	3	0.051	6.2	3	0.7	Mild			
Pyridine	110-86-1	liquid	100%	>99.9	1	73.7			4.468			140.7			Very severe	Severe	Cat I	Balls et al. (1995)
Pyridine	110-86-1	liquid	100%	>99.9	2	83.7			4.117			145.4			Very severe			Balls et al. (1995)
Pyridine	110-86-1	liquid	100%	>99.9	3	61			4.763			132.4			Very severe			Balls et al. (1995)
Pyridine	110-86-1	liquid	100%	>99.9	4	87.33			7.445			199.02			Very severe			Balls et al. (1995)
Pyridine	110-86-1	liquid	100%	>99.9	5	74.7			3.204			122.7			Very severe			Balls et al. (1995)
Pyridine	110-86-1	liquid	100%	n.p.	1							102			Severe	Severe	Cat I	Gautheron et al. (1994)
Pyridine	110-86-1	liquid	100%	n.p.	2							123			Severe			Gautheron et al. (1994)
Pyridine	110-86-1	liquid	100%	n.p.	3							186			Severe			Gautheron et al. (1994)
Pyridine	110-86-1	liquid	100%	n.p.	4							79			Severe			Gautheron et al. (1994)
Pyridine	110-86-1	liquid	100%	n.p.	5							102			Severe			Gautheron et al. (1994)
Pyridine	110-86-1	liquid	100%	n.p.	6							77			Severe			Gautheron et al. (1994)
Pyridine	110-86-1	liquid	100%	n.p.	7							124			Severe			Gautheron et al. (1994)
Pyridine	110-86-1	liquid	100%	n.p.	8							132			Severe			Gautheron et al. (1994)
Pyridine	110-86-1	liquid	100%	n.p.	9							105			Severe			Gautheron et al. (1994)
Pyridine	110-86-1	liquid	100%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Pyridine	110-86-1	liquid	100%	n.p.	11							96			Severe			Gautheron et al. (1994)
Pyridine	110-86-1	liquid	100%	n.p.	12							115			Severe			Gautheron et al. (1994)
Q		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	13.5	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Quinacrine	69-05-6	solid	20%	n.p.	1	1			-0.047			0.3			Not Labeled	Mild	Cat III	Balls et al. (1995)
Quinacrine	69-05-6	solid	20%	n.p.	2	0.3			0.002			0.4			Not Labeled			Balls et al. (1995)
Quinacrine	69-05-6	solid	20%	n.p.	3	1.7			0.028			2.1			Not Labeled			Balls et al. (1995)
Quinacrine	69-05-6	solid	20%	n.p.	4	2.34			-0.033			1.85			Not Labeled			Balls et al. (1995)
Quinacrine	69-05-6	solid	20%	n.p.	5	2			0.07			3.1			Mild			Balls et al. (1995)
Quinacrine	69-05-6	solid	20%	n.p.	1							17			Mild	Moderate	Cat III	Gautheron et al. (1994)
Quinacrine	69-05-6	solid	20%	n.p.	2							29			Moderate			Gautheron et al. (1994)
Quinacrine	69-05-6	solid	20%	n.p.	3							8			Mild			Gautheron et al. (1994)
Quinacrine	69-05-6	solid	20%	n.p.	4							46			Moderate			Gautheron et al. (1994)
Quinacrine	69-05-6	solid	20%	n.p.	5							52			Moderate			Gautheron et al. (1994)
Quinacrine	69-05-6	solid	20%	n.p.	6							24			Mild			Gautheron et al. (1994)
Quinacrine	69-05-6	solid	20%	n.p.	7							15			Moderate			Gautheron et al. (1994)
Quinacrine	69-05-6	solid	20%	n.p.	8							18			Moderate			Gautheron et al. (1994)
Quinacrine	69-05-6	solid	20%	n.p.	9							58			Severe			Gautheron et al. (1994)
Quinacrine	69-05-6	solid	20%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Quinacrine	69-05-6	solid	20%	n.p.	11							3			Not Labeled			Gautheron et al. (1994)
Quinacrine	69-05-6	solid	20%	n.p.	12							72			Severe			Gautheron et al. (1994)
R		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	0.2	n.p.	n.p.	Not Labeled	Not Labeled	Cat IV	AMCP BRD
S		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	18.8	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD
Sodium hydroxide (1%)	1310-73-2	liquid	1%	reagent grade	1	100.3			4.471			167.4			Very severe	Very Severe	Cat I	Balls et al. (1995)
Sodium hydroxide (1%)	1310-73-2	liquid	1%	reagent grade	2	80.7			3.504			133.2			Very severe			Balls et al. (1995)
Sodium hydroxide (1%)	1310-73-2	liquid	1%	reagent grade	3	88.7			3.856			146.5			Very severe			Balls et al. (1995)
Sodium hydroxide (1%)	1310-73-2	liquid	1%	reagent grade	4	116.66			3.628			171.08			Very severe			Balls et al. (1995)
Sodium hydroxide (1%)	1310-73-2	liquid	1%	reagent grade	5	88			2.888			132.3			Very severe			Balls et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Sodium hydroxide (10%)	1310-73-2	liquid	10%	reagent grade	1	232.3			3.53			285.2			Very severe	Severe	Cat I	Balls et al. (1995)
Sodium hydroxide (10%)	1310-73-2	liquid	10%	reagent grade	2	173.3			3.382			224.1			Very severe			Balls et al. (1995)
Sodium hydroxide (10%)	1310-73-2	liquid	10%	reagent grade	3	197			3.849			254.7			Very severe			Balls et al. (1995)
Sodium hydroxide (10%)	1310-73-2	liquid	10%	reagent grade	4	283			4.329			348.27			Very severe			Balls et al. (1995)
Sodium hydroxide (10%)	1310-73-2	liquid	10%	reagent grade	5	197.3			3.321			247.2			Very severe			Balls et al. (1995)
Sodium hydroxide (10%)	1310-73-2	liquid	10%	n.p.	1 (1)	176.7	3	31.4	4.551	3	1.019	245.0	3	28.7	Very severe	Very Severe	Cat I	Southee (1998)
Sodium hydroxide (10%)	1310-73-2	liquid	10%	n.p.	1 (2)	172.0	3	1.7	3.676	3	0.201	227.1	3	3.4	Very severe			Southee (1998)
Sodium hydroxide (10%)	1310-73-2	liquid	10%	n.p.	2 (1)	170.0	3	20.7	4.755	3	0.586	241.3	3	11.9	Very severe			Southee (1998)
Sodium hydroxide (10%)	1310-73-2	liquid	10%	n.p.	2 (2)	166.7	3	12.6	4.590	3	0.405	235.5	3	7.3	Very severe			Southee (1998)
Sodium hydroxide (10%)	1310-73-2	liquid	10%	n.p.	3 (1)	124.0	3	13.7	4.604	3	0.380	193.1	3	19.0	Very severe			Southee (1998)
Sodium hydroxide (10%)	1310-73-2	liquid	10%	n.p.	3 (2)	165.3	3	21.2	3.303	3	0.388	214.9	3	15.5	Very severe			Southee (1998)
Sodium lauryl sulfate (15 %)	151-21-3	liquid	15%	98	1	4			2.884			47.3			Moderate	Mild	Cat II	Balls et al. (1995)
Sodium lauryl sulfate (15 %)	151-21-3	liquid	15%	98	2	6			5.801			93			Very severe			Balls et al. (1995)
Sodium lauryl sulfate (15 %)	151-21-3	liquid	15%	98	3	3.3			3.988			63.2			Severe			Balls et al. (1995)
Sodium lauryl sulfate (15 %)	151-21-3	liquid	15%	98	4	1.66			3.862			59.61			Severe			Balls et al. (1995)
Sodium lauryl sulfate (15 %)	151-21-3	liquid	15%	98	5	7.7			3.042			53.3			Moderate			Balls et al. (1995)
Sodium lauryl sulfate (15%)	151-21-3	liquid	15%	98	1 (1)	-0.8	3	0.0	0.408	3	0.024	5.4	3	0.4	Mild	Mild	Cat III	Southee (1998)
Sodium lauryl sulfate (15%)	151-21-3	liquid	15%	98	1 (2)	0.0	3	0.6	0.348	3	0.182	5.2	3	2.7	Mild			Southee (1998)
Sodium lauryl sulfate (15%)	151-21-3	liquid	15%	98	2 (1)	0.7	3	1.0	1.012	3	0.461	15.9	3	7.6	Mild			Southee (1998)
Sodium lauryl sulfate (15%)	151-21-3	liquid	15%	98	2 (2)	1.0	3	0.6	1.086	3	0.083	17.3	3	1.7	Mild			Southee (1998)
Sodium lauryl sulfate (15%)	151-21-3	liquid	15%	98	3 (1)	0.7	3	0.6	0.518	3	0.11	8.7	3	1.4	Mild			Southee (1998)
Sodium lauryl sulfate (15%)	151-21-3	liquid	15%	98	3 (2)	1.3	3	0.6	0.283	3	0.064	5.6	3	1.5	Mild			Southee (1998)
Sodium lauryl sulfate (3 %)	151-21-3	liquid	3%	98	1	12.3			1.29			31.7			Moderate	Moderate	Cat II	Balls et al. (1995)
Sodium lauryl sulfate (3 %)	151-21-3	liquid	3%	98	2	3.3			1.892			31.7			Moderate			Balls et al. (1995)
Sodium lauryl sulfate (3 %)	151-21-3	liquid	3%	98	3	0.3			1.801			27.3			Moderate			Balls et al. (1995)
Sodium lauryl sulfate (3 %)	151-21-3	liquid	3%	98	4	6			1.348			26.22			Moderate			Balls et al. (1995)
Sodium lauryl sulfate (3 %)	151-21-3	liquid	3%	98	5	0			0.82			12.3			Mild			Balls et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Sodium oxalate	62-76-0	solid	20%	>99	1	1.3			0.054			2.1			Not Labeled	Mild	Cat III	Balls et al. (1995)
Sodium oxalate	62-76-0	solid	20%	>99	2	6.7			0.059			7.6			Mild			Balls et al. (1995)
Sodium oxalate	62-76-0	solid	20%	>99	3	3			0.187			5.8			Mild			Balls et al. (1995)
Sodium oxalate	62-76-0	solid	20%	>99	4	43			0.556			49.59			Moderate			Balls et al. (1995)
Sodium oxalate	62-76-0	solid	20%	>99	5	4			0.081			4.9			Mild			Balls et al. (1995)
Sodium oxalate	62-76-0	solid	20%	n.p.	1							2			Not Labeled	Mild	Cat III	Gautheron et al. (1994)
Sodium oxalate	62-76-0	solid	20%	n.p.	2							2			Not Labeled			Gautheron et al. (1994)
Sodium oxalate	62-76-0	solid	20%	n.p.	3							9			Mild			Gautheron et al. (1994)
Sodium oxalate	62-76-0	solid	20%	n.p.	4							5			Mild			Gautheron et al. (1994)
Sodium oxalate	62-76-0	solid	20%	n.p.	5							3			Not Labeled			Gautheron et al. (1994)
Sodium oxalate	62-76-0	solid	20%	n.p.	6							2			Not Labeled			Gautheron et al. (1994)
Sodium oxalate	62-76-0	solid	20%	n.p.	7							4			Mild			Gautheron et al. (1994)
Sodium oxalate	62-76-0	solid	20%	n.p.	8							3			Not Labeled			Gautheron et al. (1994)
Sodium oxalate	62-76-0	solid	20%	n.p.	9							3			Not Labeled			Gautheron et al. (1994)
Sodium oxalate	62-76-0	solid	20%	n.p.	10							9			Mild			Gautheron et al. (1994)
Sodium oxalate	62-76-0	solid	20%	n.p.	11							11			Mild			Gautheron et al. (1994)
Sodium oxalate	62-76-0	solid	20%	n.p.	12							4			Mild			Gautheron et al. (1994)
Sodium oxalate	62-76-0	solid	20%	99	1 (1)	8.4	3	1.2	0.128	3	0.16	10.3	3	1.4	Mild	Mild	Cat III	Southee (1998)
Sodium oxalate	62-76-0	solid	20%	99	1 (2)	3.4	3	0.6	0.071	3	0.03	4.4	3	1.0	Mild			Southee (1998)
Sodium oxalate	62-76-0	solid	20%	99	2 (1)	-1.0	3	1.7	0.05	3	0.054	-0.3	3	1.5	Not Labeled			Southee (1998)
Sodium oxalate	62-76-0	solid	20%	99	2 (2)	-1.0	3	2.1	0.055	3	0.012	-0.1	3	2.1	Not Labeled			Southee (1998)
Sodium oxalate	62-76-0	solid	20%	99	3 (1)	2.0	3	0.6	0.051	3	0.032	2.7	3	0.9	Not Labeled			Southee (1998)
Sodium oxalate	62-76-0	solid	20%	99	3 (2)	2.3	3	1.0	0.15	3	0.022	4.5	3	1.3	Mild			Southee (1998)
Sodium perborate	10486-00-7	solid	20%	98.6	1	10			8.908			143.6			Very severe	Very Severe	Cat I	Balls et al. (1995)
Sodium perborate	10486-00-7	solid	20%	98.6	2	13.7			6.982			118.4			Very severe			Balls et al. (1995)
Sodium perborate	10486-00-7	solid	20%	98.6	3	10			5.749			96.2			Very severe			Balls et al. (1995)
Sodium perborate	10486-00-7	solid	20%	98.6	4	11			3.568			64.531			Severe			Balls et al. (1995)
Sodium perborate	10486-00-7	solid	20%	98.6	5	9.7			3.547			62.9			Severe			Balls et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
T		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	1.8	n.p.	n.p.	Not Labeled	Not Labeled	Cat IV	AMCP BRD
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	97	1	24			-0.023			23.6			Mild	Mild	Cat III	Balls et al. (1995)
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	97	2	8.3			-0.027			7.9			Mild			Balls et al. (1995)
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	97	3	14.3			-0.008			14.2			Mild			Balls et al. (1995)
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	97	4	21.33			-0.045			20.65			Mild			Balls et al. (1995)
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	97	5	6			0.19			8.9			Mild			Balls et al. (1995)
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	n.p.	1							5			Mild	Mild	Cat III	Gautheron et al. (1994)
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	n.p.	2							1			Not Labeled			Gautheron et al. (1994)
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	n.p.	3							2			Not Labeled			Gautheron et al. (1994)
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	n.p.	4							6			Mild			Gautheron et al. (1994)
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	n.p.	5							0			Not Labeled			Gautheron et al. (1994)
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	n.p.	6							4			Mild			Gautheron et al. (1994)
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	n.p.	7							2			Not Labeled			Gautheron et al. (1994)
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	n.p.	8							19			Mild			Gautheron et al. (1994)
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	n.p.	9							3			Not Labeled			Gautheron et al. (1994)
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	n.p.	11							18			Mild			Gautheron et al. (1994)
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	n.p.	12							6			Mild			Gautheron et al. (1994)
Thiadiazole alkyl derivative	-	liquid	100%	n.p.	-	7.3			0.237			10.9			Moderate	Moderate	Cat III	Bailey et al. (2004)
Thiourea	62-56-6	solid	20%	>99	1	88			4.095			149.4			Very severe	Severe	Cat I	Balls et al. (1995)
Thiourea	62-56-6	solid	20%	>99	2	106.3			2.19			139.2			Very severe			Balls et al. (1995)
Thiourea	62-56-6	solid	20%	>99	3	82			3.572			135.6			Very severe			Balls et al. (1995)
Thiourea	62-56-6	solid	20%	>99	4	81.01			3.76			137.44			Very severe			Balls et al. (1995)
Thiourea	62-56-6	solid	20%	>99	5	74			1.671			99.1			Very severe			Balls et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Thiourea	62-56-6	solid	20%	n.p.	1							146			Severe	Severe	Cat I	Gautheron et al. (1994)
Thiourea	62-56-6	solid	20%	n.p.	2							175			Severe			Gautheron et al. (1994)
Thiourea	62-56-6	solid	20%	n.p.	3							169			Severe			Gautheron et al. (1994)
Thiourea	62-56-6	solid	20%	n.p.	4							152			Severe			Gautheron et al. (1994)
Thiourea	62-56-6	solid	20%	n.p.	5							140			Severe			Gautheron et al. (1994)
Thiourea	62-56-6	solid	20%	n.p.	6							120			Severe			Gautheron et al. (1994)
Thiourea	62-56-6	solid	20%	n.p.	7							129			Severe			Gautheron et al. (1994)
Thiourea	62-56-6	solid	20%	n.p.	8							173			Severe			Gautheron et al. (1994)
Thiourea	62-56-6	solid	20%	n.p.	9							151			Severe			Gautheron et al. (1994)
Thiourea	62-56-6	solid	20%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Thiourea	62-56-6	solid	20%	n.p.	11							203			Severe			Gautheron et al. (1994)
Thiourea	62-56-6	solid	20%	n.p.	12							104			Severe			Gautheron et al. (1994)
Toilet Bowl Cleaner (#1)	-	liquid	100%	n.p.	-	8.700	5		0.323	5		13.5	5		Mild	Mild	Cat III	Swanson et al. (1995)
Toilet Bowl Cleaner (#4)	-	liquid	100%	n.p.	-	10.5	5		0.303	5		15	5		Mild	Mild	Cat III	Swanson et al. (1995)
Toluene	108-88-3	liquid	100%	99	1	9.3			2.26			43.3			Moderate	Moderate	Cat II	Balls et al. (1995)
Toluene	108-88-3	liquid	100%	99	2	6			1.813			33.2			Moderate			Balls et al. (1995)
Toluene	108-88-3	liquid	100%	99	3	5.3			2.122			37.2			Moderate			Balls et al. (1995)
Toluene	108-88-3	liquid	100%	99	4	2			2.427			38.41			Moderate			Balls et al. (1995)
Toluene	108-88-3	liquid	100%	99	5	4			1.473			26.1			Moderate			Balls et al. (1995)
Trichloroacetic acid (3%)	76-03-9	liquid	3%	reagent grade	1	79.3			0.173			81.9			Very severe	Severe/Very Severe	Cat I	Balls et al. (1995)
Trichloroacetic acid (3%)	76-03-9	liquid	3%	reagent grade	2	49			0.053			49.8			Moderate			Balls et al. (1995)
Trichloroacetic acid (3%)	76-03-9	liquid	3%	reagent grade	3	73.7			0.111			75.3			Severe			Balls et al. (1995)
Trichloroacetic acid (3%)	76-03-9	liquid	3%	reagent grade	4	92.33			0.042			92.97			Very severe			Balls et al. (1995)
Trichloroacetic acid (3%)	76-03-9	liquid	3%	reagent grade	5	78.4			0.067			79.3			Severe			Balls et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Trichloroacetic acid (30%)	76-03-9	liquid	30%	reagent grade	1	228			2.93			272			Very severe	Very Severe	Cat I	Balls et al. (1995)
Trichloroacetic acid (30%)	76-03-9	liquid	30%	reagent grade	2	154.7			4.687			225			Very severe			Balls et al. (1995)
Trichloroacetic acid (30%)	76-03-9	liquid	30%	reagent grade	3	245.3			3.44			296.9			Very severe			Balls et al. (1995)
Trichloroacetic acid (30%)	76-03-9	liquid	30%	reagent grade	4	277			3.072			323.08			Very severe			Balls et al. (1995)
Trichloroacetic acid (30%)	76-03-9	liquid	30%	reagent grade	5	157			3.115			203.7			Very severe			Balls et al. (1995)
Triethanolamine	102-71-6	liquid	100%	n.p.	1							2			Not Labeled	Not Labeled	Cat IV	Gautheron et al. (1994)
Triethanolamine	102-71-6	liquid	100%	n.p.	2							4			Mild			Gautheron et al. (1994)
Triethanolamine	102-71-6	liquid	100%	n.p.	3							0			Not Labeled			Gautheron et al. (1994)
Triethanolamine	102-71-6	liquid	100%	n.p.	4							0			Not Labeled			Gautheron et al. (1994)
Triethanolamine	102-71-6	liquid	100%	n.p.	5							-1			Not Labeled			Gautheron et al. (1994)
Triethanolamine	102-71-6	liquid	100%	n.p.	6							1			Not Labeled			Gautheron et al. (1994)
Triethanolamine	102-71-6	liquid	100%	n.p.	7							1			Not Labeled			Gautheron et al. (1994)
Triethanolamine	102-71-6	liquid	100%	n.p.	8							3			Not Labeled			Gautheron et al. (1994)
Triethanolamine	102-71-6	liquid	100%	n.p.	9							3			Not Labeled			Gautheron et al. (1994)
Triethanolamine	102-71-6	liquid	100%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Triethanolamine	102-71-6	liquid	100%	n.p.	11							5			Mild			Gautheron et al. (1994)
Triethanolamine	102-71-6	liquid	100%	n.p.	12							6			Mild			Gautheron et al. (1994)
Triton X-100 (10%)	9002-93-1	liquid	10%	98	1	6			5.312			85.7			Very severe	Severe/Very Severe	Cat I	Balls et al. (1995)
Triton X-100 (10%)	9002-93-1	liquid	10%	98	2	6.7			4.624			76			Severe			Balls et al. (1995)
Triton X-100 (10%)	9002-93-1	liquid	10%	98	3	6			5.337			86.1			Very severe			Balls et al. (1995)
Triton X-100 (10%)	9002-93-1	liquid	10%	98	4	3.33			3.617			57.58			Severe			Balls et al. (1995)
Triton X-100 (10%)	9002-93-1	liquid	10%	98	5	7.7			2.567			46.2			Moderate			Balls et al. (1995)
Triton X-100 (5 %)	9002-93-1	liquid	5%	98	1	5.3			4.6			74.3			Severe	Severe	Cat I	Balls et al. (1995)
Triton X-100 (5 %)	9002-93-1	liquid	5%	98	2	8.3			6.553			106.6			Very severe			Balls et al. (1995)
Triton X-100 (5 %)	9002-93-1	liquid	5%	98	3	3.7			5.099			80.2			Very severe			Balls et al. (1995)
Triton X-100 (5 %)	9002-93-1	liquid	5%	98	4	5			4.79			76.79			Very severe			Balls et al. (1995)
Triton X-100 (5 %)	9002-93-1	liquid	5%	98	5	7.7			3.06			53.6			Moderate			Balls et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Triton X-100 (5%)	9002-93-1	liquid	5%	98	1 (1)	3.3	3	1.0	0.023	3	0.004	3.7	3	1.1	Mild	Mild	Cat III	Southee (1998)
Triton X-100 (5%)	9002-93-1	liquid	5%	98	1 (2)	1.3	3	1.0	0.035	3	0.006	1.8	3	1.0	Not Labeled			Southee (1998)
Triton X-100 (5%)	9002-93-1	liquid	5%	98	2 (1)	1.4	3	0.6	0.298	3	0.123	5.8	3	2.4	Mild			Southee (1998)
Triton X-100 (5%)	9002-93-1	liquid	5%	98	2 (2)	0.0	3	0.6	0.226	3	0.086	3.4	3	1.0	Mild			Southee (1998)
Triton X-100 (5%)	9002-93-1	liquid	5%	98	3 (1)	2.7	3	1.0	0.023	3	0.009	3.0	3	1.1	Not Labeled			Southee (1998)
Triton X-100 (5%)	9002-93-1	liquid	5%	98	3 (2)	1.4	3	0.6	0.038	3	0.013	1.9	3	0.6	Not Labeled			Southee (1998)
Triton X-155	9010-44-0	surfactant	10%	n.p.	1							-1			Not Labeled	Not Labeled	Cat IV	Gautheron et al. (1994)
Triton X-155	9010-44-0	surfactant	10%	n.p.	2							1			Not Labeled			Gautheron et al. (1994)
Triton X-155	9010-44-0	surfactant	10%	n.p.	3							-1			Not Labeled			Gautheron et al. (1994)
Triton X-155	9010-44-0	surfactant	10%	n.p.	4							0			Not Labeled			Gautheron et al. (1994)
Triton X-155	9010-44-0	surfactant	10%	n.p.	5							2			Not Labeled			Gautheron et al. (1994)
Triton X-155	9010-44-0	surfactant	10%	n.p.	6							2			Not Labeled			Gautheron et al. (1994)
Triton X-155	9010-44-0	surfactant	10%	n.p.	7							0			Not Labeled			Gautheron et al. (1994)
Triton X-155	9010-44-0	surfactant	10%	n.p.	8							2			Not Labeled			Gautheron et al. (1994)
Triton X-155	9010-44-0	surfactant	10%	n.p.	9							3			Not Labeled			Gautheron et al. (1994)
Triton X-155	9010-44-0	surfactant	10%	n.p.	10							no data			n.a.			Gautheron et al. (1994)
Triton X-155	9010-44-0	surfactant	10%	n.p.	11							-2			Not Labeled			Gautheron et al. (1994)
Triton X-155	9010-44-0	surfactant	10%	n.p.	12							0			Not Labeled			Gautheron et al. (1994)
Tween 20	9005-64-5	liquid	n.p.	98	1	-0.7			0.006			-0.6			Not Labeled	Not Labeled	Cat IV	Balls et al. (1995)
Tween 20	9005-64-5	liquid	n.p.	98	2	-0.3			-0.052			-1.1			Not Labeled			Balls et al. (1995)
Tween 20	9005-64-5	liquid	n.p.	98	3	-2			0.026			-1.6			Not Labeled			Balls et al. (1995)
Tween 20	9005-64-5	liquid	n.p.	98	4	2.67			0.0003			2.711			Not Labeled			Balls et al. (1995)
Tween 20	9005-64-5	liquid	n.p.	98	5	0.1			0.026			0.4			Not Labeled			Balls et al. (1995)

In Vitro Data for Substances Tested in the BCOP Assay: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	Lab No.	Mean Opacity	n	SD - Opacity	Mean OD_{490}	n	SD - OD_{490}	*In Vitro* Score	n	SD - Score	*In Vitro* Classification[1]	*In Vitro* Consensus Classification Severe ≥ 55.1[2]	*In Vitro* Consensus Classification Severe ≥ 75[2]	Reference
Tween 20	9005-64-5	liquid	100%	98	1 (1)	0.3	3	0.0	0.003	3	0.012	0.3	3	0.2	Not Labeled	Not Labeled	Not Labeled	Southee (1998)
Tween 20	9005-64-5	liquid	100%	98	1 (2)	0.0	3	1.5	0.004	3	0.01	0.0	3	1.6	Not Labeled			Southee (1998)
Tween 20	9005-64-5	liquid	100%	98	2 (1)	0.4	3	0.6	0.001	3	0.002	0.4	3	0.6	Not Labeled			Southee (1998)
Tween 20	9005-64-5	liquid	100%	98	2 (2)	0.4	3	0.6	0.003	3	0.008	0.4	3	0.5	Not Labeled			Southee (1998)
Tween 20	9005-64-5	liquid	100%	98	3 (1)	0.0	3	0.0	0.022	3	0.018	0.3	3	0.3	Not Labeled			Southee (1998)
Tween 20	9005-64-5	liquid	100%	98	3 (2)	0.0	3	1.0	0.001	3	0.022	0.0	3	1.3	Not Labeled			Southee (1998)
U		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	3.4	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD
V		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	20.8	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD
W		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	5.7	n.p.	n.p.	Cat III	Cat III	Cat III	AMCP BRD
X		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	81.9	n.p.	n.p.	Cat I	Cat I	Cat I	AMCP BRD
Y		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	74.9	n.p.	n.p.	Cat I	Cat I	Cat II	AMCP BRD
Z		n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	31.6	n.p.	n.p.	Cat II	Cat II	Cat II	AMCP BRD

Abbreviations: CASRN = Chemical Abstract Services Registry Number; n.a. = Not Applicable; n.p. = Not Provided; SCNM = Study Criteria Not Met.

[1] *In Vitro* Classification represents the BCOP ocular irritancy classification assigned for each chemical in the study for each test for a specific substance

[2] Consensus classification represents the overall BCOP ocular irritancy classification assigned for each chemical in the study based on the majority of ocular irritancy classification calls

This page intentionally left blank

Annex III

Comparison of *In Vivo* and *In Vitro* Ocular Irritancy Classifications

Annex III-1
BCOP Data Sorted by Reference.....C-269

Annex III-2
BCOP Data Sorted by Substance Name.....C-283

This page intentionally left blank

Annex III-1

BCOP Data Sorted by Reference

This page intentionally left blank

Comparison of In Vivo and In Vitro Ocular Irritancy Classifications: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	*In Vitro* Consensus Classification Severe ≥ 55.1[1]	*In Vivo* GHS[2,3]	*In Vivo* EPA[4,5]	*In Vivo* EU[6,7]	FHSA-20%[8]	FHSA-67%[9]	*In Vitro* Consensus Classification Severe ≥ 75[1]	Reference
A		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
AB		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
AC		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
AD		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
AE		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat II	AMCP BRD
AF		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category IV	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD
AG		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Inconclusive	Cat I	AMCP BRD
AH		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	SCNM	SCNM	Cat I	AMCP BRD
AI		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
AJ		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
AK		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
AL		n.p.	n.p.	n.p.	Cat I	Category 2A	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
AM		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
AN		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Not labeled	Not labeled	Cat I	AMCP BRD
AO		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
AP		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
AQ		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
AR		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
AS		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
AT		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
AU		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD

Comparison of In Vivo and In Vitro Ocular Irritancy Classifications: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	*In Vitro* Consensus Classification Severe ≥ 55.1[1]	*In Vivo* GHS[2,3]	*In Vivo* EPA[4,5]	*In Vivo* EU[6,7]	FHSA-20%[8]	FHSA-67%[9]	*In Vitro* Consensus Classification Severe ≥ 75[1]	Reference
AV		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	SCNM	SCNM	Cat I	AMCP BRD
AW		n.p.	n.p.	n.p.	Cat II	Category 1	Category I	n.p.	Irritant	Irritant	Cat II	AMCP BRD
AX		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
AY		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
B		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
BB		n.p.	n.p.	n.p.	Cat III	SCNM	Category IV	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD
BD		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category III	n.p.	Irritant	Irritant	Cat III	AMCP BRD
BE		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category III	n.p.	Irritant	Inconclusive	Cat III	AMCP BRD
BF		n.p.	n.p.	n.p.	Cat I	Category 2A	Category III	n.p.	Irritant	Irritant	Cat II	AMCP BRD
BJ		n.p.	n.p.	n.p.	Cat I	Nonirritant	Category III	n.p.	Irritant	Irritant	Cat I	AMCP BRD
BJ		n.p.	n.p.	n.p.	Cat II	Nonirritant	Category III	n.p.	Irritant	Irritant	Cat II	AMCP BRD
BK		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category III	n.p.	Irritant	Irritant	Cat III	AMCP BRD
BL		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category IV	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD
BM		n.p.	n.p.	n.p.	Cat II	Nonirritant	Category IV	n.p.	Not labeled	Not labeled	Cat II	AMCP BRD
BN		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category IV	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD
BP		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category IV	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD
BQ		n.p.	n.p.	n.p.	Cat II	Nonirritant	Category IV	n.p.	Not labeled	Not labeled	Cat II	AMCP BRD
C		n.p.	n.p.	n.p.	Cat II	Category 1	Category I	n.p.	Irritant	Irritant	Cat II	AMCP BRD
CG		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category IV	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD
CH		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category III	n.p.	Irritant	Inconclusive	Cat III	AMCP BRD
D		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD

Comparison of In Vivo and In Vitro Ocular Irritancy Classifications: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	*In Vitro* Consensus Classification Severe ≥ 55.1[1]	*In Vivo* GHS[2,3]	*In Vivo* EPA[4,5]	*In Vivo* EU[6,7]	FHSA-20%[8]	FHSA-67%[9]	*In Vitro* Consensus Classification Severe ≥ 75[1]	Reference
E		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	SCNM	SCNM	Cat I	AMCP BRD
EF		n.p.	n.p.	n.p.	Cat I	Category 2A	Category II	n.p.	Irritant	Irritant	Cat I	AMCP BRD
EG		n.p.	n.p.	n.p.	Cat I	Category 2A	Category II	n.p.	Irritant	Irritant	Cat II	AMCP BRD
F		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
G		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
H		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category II	n.p.	Irritant	Irritant	Cat III	AMCP BRD
I		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category III	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD
J		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category III	n.p.	Irritant	Irritant	Cat III	AMCP BRD
K		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category IV	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD
L		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category III	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD
M		n.p.	n.p.	n.p.	Cat I	Nonirritant	Category III	n.p.	Irritant	Irritant	Cat II	AMCP BRD
N		n.p.	n.p.	n.p.	Cat I	Nonirritant	Category III	n.p.	Irritant	Inconclusive	Cat I	AMCP BRD
O		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category IV	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD
P		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category IV	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD
Q		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category IV	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD
R		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category IV	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD
S		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category IV	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD
T		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category IV	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD
U		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category IV	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD
V		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category IV	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD
W		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category IV	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD

Comparison of In Vivo and In Vitro Ocular Irritancy Classifications: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	*In Vitro* Consensus Classification Severe ≥ 55.1[1]	*In Vivo* GHS[2,3]	*In Vivo* EPA[4,5]	*In Vivo* EU[6,7]	FHSA-20%[8]	FHSA-67%[9]	*In Vitro* Consensus Classification Severe ≥ 75[1]	Reference
X		n.p.	n.p.	n.p.	Cat I	Category 2A	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
Y		n.p.	n.p.	n.p.	Cat I	Category 2A	Category II	n.p.	Irritant	Irritant	Cat II	AMCP BRD
Z		n.p.	n.p.	n.p.	Cat II	Category 2A	Category II	n.p.	Irritant	Irritant	Cat II	AMCP BRD
2-Chloro-2,4,4-trimethylpentane	-	liquid	100%	n.p.	Mild	Nonirritant	Category IV	Nonirritant	Irritant	Irritant	Cat III	Bailey et al. (2004)
5-Ethylidene-2-norbornene	16219-75-3	liquid	100%	n.p.	Mild	Nonirritant	Category IV	Nonirritant			Cat III	Bailey et al. (2004)
Alkyl phosphoric acid ester/amine salt	-	liquid	100%	n.p.	Severe	Category 1	SCNM	R41	Irritant	Irritant	Cat I	Bailey et al. (2004)
Aromatic hydrocarbon #1	-	liquid	100%	n.p.	Nonirritant	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Bailey et al. (2004)
Aromatic hydrocarbon #2	-	liquid	100%	n.p.	Mild	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Bailey et al. (2004)
Aryl phosponates	-	liquid	100%	n.p.	Moderate	Category 2B	SCNM	SCNM	Irritant	Irritant	Cat II	Bailey et al. (2004)
Carboxylic acid amides	-	solid	100%	n.p.	Moderate	Category 1	Category I	R41	Irritant	Irritant	Cat II	Bailey et al. (2004)
Clarified slurry oil	-	liquid	100%	n.p.	Mild	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Bailey et al. (2004)
Cutting fluid (conc.) #1	-	liquid	100%	n.p.	Mild	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Bailey et al. (2004)
Cutting fluid (conc.) #2	-	liquid	100%	n.p.	Mild	Nonirritant	Category III	Nonirritant	Irritant	Inconclusive	Cat III	Bailey et al. (2004)
Ethylhexyl acid phosphate ester	-	liquid	100%	n.p.	Severe	Category 1	SCNM	R41	Irritant	Irritant	Cat I	Bailey et al. (2004)
Methyl cyclopentadiene dimer	-	liquid	100%	n.p.	Nonirritant	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Bailey et al. (2004)
Petroleum wax	-	solid	100%	n.p.	Nonirritant	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Bailey et al. (2004)
Polyalkenylsuccinate ester/amine salt	-	liquid	100%	n.p.	Nonirritant	SCNM	Category III	SCNM	Irritant	Irritant	Cat III	Bailey et al. (2004)
Process oil	-	liquid	100%	n.p.	Nonirritant	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Bailey et al. (2004)
Thiadiazole alkyl derivative	-	liquid	100%	n.p.	Moderate	SCNM	Category III	SCNM	Irritant	Irritant	Cat III	Bailey et al. (2004)
1-Naphthalene acetic acid	86-87-3	solid	20%	96	Very Severe	Category 1	Category I	SCNM	Irritant	Irritant	Cat II	Balls et al. (1995)
1-Naphthalene acetic acid, Na salt	61-31-4	solid	20%	95	Very Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Balls et al. (1995)

Comparison of In Vivo and In Vitro Ocular Irritancy Classifications: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	*In Vitro* Consensus Classification Severe ≥ 55.1[1]	*In Vivo* GHS[2,3]	*In Vivo* EPA[4,5]	*In Vivo* EU[6,7]	FHSA-20%[8]	FHSA-67%[9]	*In Vitro* Consensus Classification Severe ≥ 75[1]	Reference
2,2-Dimethylbutanoic acid	595-37-9	liquid	100%	96	Very Severe	SCNM	Category I	SCNM	Irritant	Irritant	Cat I	Balls et al. (1995)
2,5-Dimethylhexanediol	110-03-2	solid	20%	99.5	Mild	Category 1	Category I	R41	Irritant	Irritant	Cat III	Balls et al. (1995)
2,6-Dichlorobenzoyl chloride	4659-45-4	liquid	100%	99	Mild	Category 2A	Category II	SCNM	Irritant	Irritant	Cat III	Balls et al. (1995)
2-Ethyl-1-hexanol	104-76-7	liquid	100%	99	Moderate	Category 2A	Category II	R36	Irritant	Irritant	Cat II	Balls et al. (1995)
4-Carboxybenzaldehyde	619-66-9	solid	20%	95	Severe	Category 2A	Category II	R36	Irritant	Irritant	Cat II	Balls et al. (1995)
Acetone	67-64-1	liquid	100%	99	Very Severe	Category 2A	Category II	R36	Irritant	Irritant	Cat I	Balls et al. (1995)
Ammonium nitrate	6484-52-2	solid	20%	>99.9	Mild	Category 2B	Category III	R36	Irritant	Irritant	Cat III	Balls et al. (1995)
Benzalkonium chloride (1 %)	8001-54-5	liquid	1%	98	Very Severe	Category 1	Category II	R41	Irritant	Irritant	Cat I	Balls et al. (1995)
Benzalkonium chloride (10%)	8001-54-5	liquid	10%	98	Very Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Balls et al. (1995)
Benzalkonium chloride (5%)	8001-54-5	liquid	5%	98	Very Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Balls et al. (1995)
Benzoyl-L-tartaric acid	2743-38-6	solid	20%		Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Balls et al. (1995)
Butyl acetate	123-86-4	liquid	100%	99	Moderate	Nonirritant	Category III	Nonirritant	Irritant	Inconclusive	Cat II	Balls et al. (1995)
Captan 90 concentrate	133-06-2	solid	20%	90	Moderate	Category 1	Category I	R41	Irritant	Irritant	Cat II	Balls et al. (1995)
Cetylpyridinium bromide (0.1%)	140-72-7	liquid	0%	98	Mild	Nonirritant	Category III	Nonirritant	Irritant	Inconclusive	Cat III	Balls et al. (1995)
Cetylpyridinium bromide (10%)	140-72-7	liquid	10%	98	Very Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Balls et al. (1995)
Cetylpyridinium bromide (6%)	140-72-7	liquid	6%	98	Severe	Category 1	SCNM	R41	Irritant	Irritant	Cat II	Balls et al. (1995)
Chlorhexidine	55-56-1	solid	20%	n.p.	Very Severe	Category 1	SCNM	SCNM	Irritant	Irritant	Cat I	Balls et al. (1995)
Cyclohexanol	108-93-0	liquid	100%	97	Moderate	Category 1	Category I	R41	Irritant	Irritant	Cat II	Balls et al. (1995)
Dibenzyl phosphate	1623-08-1	solid	0.2	99	Very Severe	Category 2A	Category II	R36	Irritant	Irritant	Cat I	Balls et al. (1995)
Ethanol	64-17-5	liquid	100%	n.p.	Severe	Category 2A	Category III	Nonirritant	Irritant	Irritant	Cat I	Balls et al. (1995)
Ethyl acetate	141-78-6	liquid	1	99	Moderate	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat II	Balls et al. (1995)
Ethyl trimethyl acetate	3938-95-2	liquid	1	99	Mild	Nonirritant	Category III	Nonirritant	Not labeled	Not labeled	Cat III	Balls et al. (1995)

Comparison of In Vivo and In Vitro Ocular Irritancy Classifications: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	*In Vitro* Consensus Classification Severe ≥ 55.1[1]	*In Vivo* GHS[2,3]	*In Vivo* EPA[4,5]	*In Vivo* EU[6,7]	FHSA-20%[8]	FHSA-67%[9]	*In Vitro* Consensus Classification Severe ≥ 75[1]	Reference
Ethyl-2-methylacetoacetate	609-14-3	liquid	100%	97	Mild	Category 2B	Category III	Nonirritant	Irritant	Irritant	Cat III	Balls et al. (1995)
Fomesafen	72128-02-0	solid	20%	97.5	Severe	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat II	Balls et al. (1995)
gamma-Butyrolactone	96-48-0	liquid	100%	>99	Severe	Category 2A	Category II	R36	Irritant	Irritant	Cat II	Balls et al. (1995)
Glycerol	56-81-5	liquid	100%	>99.5	Nonirritant	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Balls et al. (1995)
Imidazole	288-32-4	solid	20%	99	Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Balls et al. (1995)
Isobutanol	78-83-1	liquid	100%	99.9	Moderate	Category 2A	Category II	R36	Irritant	Irritant	Cat II	Balls et al. (1995)
Isopropanol	67-63-0	liquid	100%	99.9	Severe	Category 2A	Category III	SCNM	Irritant	Irritant	Cat II	Balls et al. (1995)
L-Aspartic acid	70-47-3	solid	20%	100	Nonirritant	SCNM	SCNM	SCNM	Irritant	Irritant	Cat III	Balls et al. (1995)
Maneb	12427-38-2	solid	20%	90	Mild	SCNM	Category III	SCNM	Irritant	Irritant	Cat II	Balls et al. (1995)
Methyl acetate	79-20-9	liquid	100%	98	Moderate	Category 2A	Category II	R36	Irritant	Irritant	Cat II	Balls et al. (1995)
Methyl cyanoacetate	105-34-0	liquid	1	99	Mild	Category 2A	Category II	R36	Irritant	Irritant	Cat III	Balls et al. (1995)
Methyl ethyl ketone	78-93-3	liquid	100%	99	Severe	Category 2A	Category III	R36	Irritant	Irritant	Cat II	Balls et al. (1995)
Methyl isobutyl ketone	108-10-1	liquid	1	98	Mild	Nonirritant	Category III	Nonirritant	Irritant	Inconclusive	Cat III	Balls et al. (1995)
Methylcyclopentane	96-37-7	liquid	100%	>99	Nonirritant	Nonirritant	Category III	Nonirritant	Not labeled	Not labeled	Cat III	Balls et al. (1995)
n-Hexanol	111-27-3	liquid	100%	98	Severe/ Very Severe	Category 2A	Category II	R36	Irritant	Irritant	Cat II	Balls et al. (1995)
n-Octanol	111-87-5	liquid	100%	>99	Moderate	Category 2B	Category III	R36	Irritant	Irritant	Cat II	Balls et al. (1995)
Parafluoraniline	371-40-4	liquid	100%	99	Moderate	SCNM	SCNM	SCNM	Irritant	Irritant	Cat II	Balls et al. (1995)
Polyethylene glycol 400	25322-68-3	liquid	100%	n.p.	Nonirritant	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Balls et al. (1995)
Potassium cyanate	590-28-3	solid	20%	97	Mild	SCNM	SCNM	SCNM	Irritant	Irritant	Cat III	Balls et al. (1995)
Promethazine hydrochloride	58-33-3	solid	20%	98	Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Balls et al. (1995)
Pyridine	110-86-1	liquid	100%	>99.9	Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Balls et al. (1995)
Quinacrine	69-05-6	solid	20%	n.p.	Mild	Category 1	Category I	R41	Irritant	Irritant	Cat III	Balls et al. (1995)

Comparison of In Vivo and In Vitro Ocular Irritancy Classifications: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	*In Vitro* Consensus Classification Severe ≥ 55.1[1]	*In Vivo* GHS[2,3]	*In Vivo* EPA[4,5]	*In Vivo* EU[6,7]	FHSA-20%[8]	FHSA-67%[9]	*In Vitro* Consensus Classification Severe ≥ 75[1]	Reference
Sodium hydroxide (1%)	1310-73-2	liquid	1%	reagent grade	Very Severe	Category 2B	Category III	R36	Irritant	Irritant	Cat I	Balls et al. (1995)
Sodium hydroxide (10%)	1310-73-2	liquid	10%	reagent grade	Severe	Category 1	Category I	R41	SCNM	SCNM	Cat I	Balls et al. (1995)
Sodium lauryl sulfate (15 %)	151-21-3	liquid	15%	98	Mild	Category 1	Category I	R36	Irritant	Irritant	Cat II	Balls et al. (1995)
Sodium lauryl sulfate (3 %)	151-21-3	liquid	3%	98	Moderate	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat II	Balls et al. (1995)
Sodium oxalate	62-76-0	solid	20%	>99	Mild	Category 1	Category I	R41	Irritant	Irritant	Cat III	Balls et al. (1995)
Sodium perborate	10486-00-7	solid	20%	98.6	Very Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Balls et al. (1995)
Tetraaminopyrimidine sulfate	5392-28-9	solid	0.2	97	Mild	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat III	Balls et al. (1995)
Thiourea	62-56-6	solid	0.2	>99	Severe	SCNM	SCNM	SCNM	Irritant	Irritant	Cat I	Balls et al. (1995)
Toluene	108-88-3	liquid	100%	99	Moderate	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat II	Balls et al. (1995)
Trichloroacetic acid (3%)	76-03-9	liquid	3%	reagent grade	Severe/Very Severe	Nonirritant	Category III	Nonirritant	Irritant	Inconclusive	Cat I	Balls et al. (1995)
Trichloroacetic acid (30%)	76-03-9	liquid	30%	reagent grade	Very Severe	Category 1	Category I	R41	SCNM	SCNM	Cat I	Balls et al. (1995)
Triton X-100 (10%)	9002-93-1	liquid	10%	98	Severe/Very Severe	Category 1	Category II	R41	Irritant	Irritant	Cat I	Balls et al. (1995)
Triton X-100 (5 %)	9002-93-1	liquid	5%	98	Severe	Category 2A	Category III	R36	Irritant	Irritant	Cat I	Balls et al. (1995)
Tween 20	9005-64-5	liquid	n.p.	98	Nonirritant	Nonirritant	Category III	Nonirritant	Irritant	Inconclusive	Cat III	Balls et al. (1995)
1,2,3-Trichloropropane	96-18-4	liquid	100%	n.p.	Moderate	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat II	Gautheron et al. (1994)
1,2,4-Trimethylbenzene	95-63-6	liquid	100.00%	n.p.	Mild	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat III	Gautheron et al. (1994)
1-Nitropropane	108-03-2	liquid	100%	n.p.	Mild	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Gautheron et al. (1994)
1-Phenyl-3-pyrazolidone	92-43-3	solid	20%	n.p.	Mild	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat III	Gautheron et al. (1994)
2,4-Dichloro-5-sulfamoylbenzoic acid	2736-23-4	solid	20%	n.p.	Mild	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat III	Gautheron et al. (1994)
2,4-Pentanedione	123-54-6	liquid	100%	n.p.	Severe	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat II	Gautheron et al. (1994)
2-Aminophenol	95-55-6	solid	20%	n.p.	Mild	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Gautheron et al. (1994)
2-Ethoxyethanol	110-80-5	liquid	100%	n.p.	Severe	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat I	Gautheron et al. (1994)

Comparison of In Vivo and In Vitro Ocular Irritancy Classifications: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	*In Vitro* Consensus Classification Severe ≥ 55.1[1]	*In Vivo* GHS[2,3]	*In Vivo* EPA[4,5]	*In Vivo* EU[6,7]	FHSA-20%[8]	FHSA-67%[9]	*In Vitro* Consensus Classification Severe ≥ 75[1]	Reference
2-Mercaptopyrimidine	1450-85-7	solid	20%	n.p.	Nonirritant	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Gautheron et al. (1994)
2-Methoxyethanol	109-86-4	liquid	100%	n.p.	Severe	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat II	Gautheron et al. (1994)
3-Glycidoxypropyltrimethoxysilane	2530-83-8	liquid	100%	n.p.	Mild	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Gautheron et al. (1994)
Allyl alcohol	107-18-6	liquid	1	n.p.	Severe	Category 2A	Category III	R36	Irritant	Irritant	Cat I	Gautheron et al. (1994)
Aluminum hydroxide	21645-51-2	solid	20%	n.p.	Mild	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat III	Gautheron et al. (1994)
Anthracene	120-12-7	solid	20%	n.p.	Nonirritant	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Gautheron et al. (1994)
Benzethonium chloride	121-54-0	surfactant	10%	n.p.	Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Gautheron et al. (1994)
Betaine monohydrate	590-47-6	solid	20%	n.p.	Mild	Nonirritant	Category III	Nonirritant	Irritant	Inconclusive	Cat III	Gautheron et al. (1994)
BRIJ-35	9002-92-0	surfactant	10%	n.p.	Nonirritant	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Gautheron et al. (1994)
Butyrolactone	96-48-0	liquid	100%	n.p.	Moderate	Category 2A	Category II	R36	Irritant	Irritant	Cat II	Gautheron et al. (1994)
Cyclohexanone	108-94-1	liquid	100%	n.p.	Severe	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat I	Gautheron et al. (1994)
Deoxycholic acid, sodium salt	302-95-4	surfactant	10%	n.p.	Severe	Category 2A	Category II	R36	Irritant	Irritant	Cat I	Gautheron et al. (1994)
Diacetone alcohol	123-42-2	liquid	100%	n.p.	Moderate	SCNM	SCNM	SCNM	Irritant	Irritant	Cat II	Gautheron et al. (1994)
Dibenzoyl-L-tartaric acid	2743-38-6	solid	20%	n.p.	Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Gautheron et al. (1994)
Dimethyl sulfoxide	67-68-5	liquid	100%	n.p.	Mild	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat III	Gautheron et al. (1994)
Dimethylbiguanide	657-24-9	solid	20%	n.p.	Nonirritant	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat III	Gautheron et al. (1994)
DL-Glutamic acid	19285-83-7	solid	20%	n.p.	Nonirritant	SCNM	SCNM	SCNM	Irritant	Inconclusive	Cat III	Gautheron et al. (1994)
Ethanol	64-17-5	liquid	100%	n.p.	Severe	SCNM	Category II	Nonirritant	Irritant	Irritant	Cat I	Gautheron et al. (1994)
EDTA, di-potassium salt	25102-12-9	solid	20%	n.p.	Nonirritant	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat III	Gautheron et al. (1994)
Ethyl acetoacetate	141-97-9	liquid	100%	n.p.	Moderate	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat II	Gautheron et al. (1994)
Furan	110-00-9	liquid	100%	n.p.	Severe	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat II	Gautheron et al. (1994)
Gluconolactone	90-80-2	solid	20%	n.p.	Severe	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat I	Gautheron et al. (1994)

Comparison of In Vivo and In Vitro Ocular Irritancy Classifications: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	*In Vitro* Consensus Classification Severe ≥ 55.1[1]	*In Vivo* GHS[2,3]	*In Vivo* EPA[4,5]	*In Vivo* EU[6,7]	FHSA-20%[8]	FHSA-67%[9]	*In Vitro* Consensus Classification Severe ≥ 75[1]	Reference
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	Moderate	Category 1	Category I	R41	Irritant	Irritant	Cat II	Gautheron et al. (1994)
Hexane	110-54-3	liquid	100%	n.p.	Nonirritant	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Gautheron et al. (1994)
Imidazole	288-32-4	solid	20%	n.p.	Severe	Category 1	Category I	R41	Irritant	Irritant		Gautheron et al. (1994)
Iminodibenzyl	494-19-9	solid	20%	n.p.	Nonirritant	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat III	Gautheron et al. (1994)
Laurylsulfobetaine	14933-08-5	surfactant	10%	n.p.	Severe	SCNM	SCNM	SCNM	Irritant	Irritant	Cat I	Gautheron et al. (1994)
Magnesium carbonate	56378-72-4	solid	20%	n.p.	Nonirritant	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat III	Gautheron et al. (1994)
Methanol	67-56-1	liquid	100%	n.p.	Severe	Nonirritant	Category II	Nonirritant	Irritant	Irritant	Cat I	Gautheron et al. (1994)
Methylisobutyl ketone	108-10-1	liquid	100%	n.p.	Mild	Nonirritant	Category III	Nonirritant	Irritant	Inconclusive	Cat III	Gautheron et al. (1994)
MYRJ-45	-	surfactant	10%	n.p.	Nonirritant	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Gautheron et al. (1994)
N-Lauroylsarcosine, sodium salt	7631-98-3	surfactant	10%	n.p.	Moderate	Category 2B	Category III	Nonirritant	Irritant	Irritant	Cat II	Gautheron et al. (1994)
Octanol	111-87-5	liquid	100%	n.p.	Moderate	Category 2B	Category III	R36	Irritant	Irritant	Cat II	Gautheron et al. (1994)
Petroleum ether	8032-32-4	liquid	1	n.p.	Mild	Nonirritant	Category III	Nonirritant	Irritant	Inconclusive	Cat III	Gautheron et al. (1994)
Phenylbutazone	50-33-9	solid	20%	n.p.	Nonirritant	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Gautheron et al. (1994)
Promethazine hydrochloride	58-33-3	solid	20%	n.p.	Severe	Category 1	Category I	R41	Irritant	Irritant	Severe	Gautheron et al. (1994)
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	n.p.	Mild	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat III	Gautheron et al. (1994)
Pyridine	110-86-1	liquid	100%	n.p.	Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Gautheron et al. (1994)
Quinacrine	69-05-6	solid	20%	n.p.	Moderate	Category 1	Category I	R41	Irritant	Irritant	Cat III	Gautheron et al. (1994)
Sodium oxalate	62-76-0	solid	20%	n.p.	Mild	Category 1	Category I	R41	Irritant	Irritant	Cat III	Gautheron et al. (1994)
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	n.p.	Mild	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat III	Gautheron et al. (1994)
Thiourea	62-56-6	solid	20%	n.p.	Severe	SCNM	SCNM	SCNM	Irritant	Irritant	Cat I	Gautheron et al. (1994)
Triethanolamine	102-71-6	liquid	100%	n.p.	Nonirritant	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Gautheron et al. (1994)
Triton X-155	9010-44-0	surfactant	10%	n.p.	Nonirritant	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Gautheron et al. (1994)

Comparison of In Vivo and In Vitro Ocular Irritancy Classifications: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	*In Vitro* Consensus Classification Severe ≥ 55.1[1]	*In Vivo* GHS[2,3]	*In Vivo* EPA[4,5]	*In Vivo* EU[6,7]	FHSA-20%[8]	FHSA-67%[9]	*In Vitro* Consensus Classification Severe ≥ 75[1]	Reference
4-Carboxybenzaldehyde	619-66-9	solid	20%	95	Moderate	Category 2A	Category II	R36	Irritant	Irritant	Cat II	Southee (1998)
Ammonium nitrate	6484-52-2	n.p.	100%	n.p.	Mild	Category 2B	Category III	R36	Irritant	Irritant	Cat III	Southee (1998)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	Very Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Southee (1998)
Ethanol	64-17-5	liquid	100%	n.p.	Moderate	Category 2A	Category III	Nonirritant	Irritant	Irritant	Cat II	Southee (1998)
Butyl cellusolve	111-76-2	liquid	100%	n.p.	Very Severe	Category 1	Category II	R41	Irritant	Irritant	Cat I	Southee (1998)
Glycerol	56-81-5	liquid	100%	>99.5	Nonirritant	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Nonirritant	Southee (1998)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	Moderate	Category 1	Category I	R41	Irritant	Irritant	Cat II	Southee (1998)
Imidazole	288-32-4	solid	20%	n.p.	Very Severe	Category 1	Category I	R41	Irritant	Irritant	Very Severe	Southee (1998)
Methyl ethyl ketone	78-93-3	liquid	100%	99	Severe	Category 2B	Category III	R36	Irritant	Irritant	Cat II	Southee (1998)
Parafluoraniline	371-40-4	liquid	100%	99	Moderate	SCNM	SCNM	SCNM	Irritant	Irritant	Cat II	Southee (1998)
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	100	Mild	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat III	Southee (1998)
Sodium hydroxide (10%)	1310-73-2	liquid	10%	n.p.	Very Severe	Category 1	Category I	R41	SCNM	SCNM	Cat I	Southee (1998)
Sodium lauryl sulfate (15%)	151-21-3	liquid	15%	98	Mild	Category 1	Category I	SCNM	Irritant	Irritant	Cat III	Southee (1998)
Sodium oxalate	62-76-0	solid	20%	99	Nonirritant	Category 1	Category I	R41	Irritant	Irritant	Cat III	Southee (1998)
Triton X-100 (5%)	9002-93-1	liquid	5%	98	Mild	Category 2B	Category III	R36	Irritant	Irritant	Cat III	Southee (1998)
Tween 20	9005-64-5	liquid	100%	98	Nonirritant	Nonirritant	Category III	Nonirritant	Irritant	Inconclusive	Nonirritant	Southee (1998)
1-1 (#1)	-	liquid	100%	n.p.	Severe	Category 2A	Category I	R36	Irritant	Irritant	Cat I	Swanson and Harbell (2000)
1-2 (#2)	-	liquid	100%	n.p.	Mild	Category 2A	Category II	R36	Irritant	Irritant	Cat III	Swanson and Harbell (2000)
1-3 (#3)	-	liquid	100%	n.p.	Moderate	Category 2A	Category II	R36	Irritant	Irritant	Cat II	Swanson and Harbell (2000)
2-4 (#4)	-	liquid	100%	n.p.	Mild	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Swanson and Harbell (2000)
2-7 (#7)	-	liquid	100%	n.p.	Moderate	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Swanson and Harbell (2000)
2-8 (#8)	-	liquid	100%	n.p.	Moderate	Nonirritant	Category III	Nonirritant	Irritant	Inconclusive	Cat II	Swanson and Harbell (2000)

Comparison of In Vivo and In Vitro Ocular Irritancy Classifications: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	*In Vitro* Consensus Classification Severe ≥ 55.1[1]	*In Vivo* GHS[2,3]	*In Vivo* EPA[4,5]	*In Vivo* EU[6,7]	FHSA-20%[8]	FHSA-67%[9]	*In Vitro* Consensus Classification Severe ≥ 75[1]	Reference
Benchmark-Group 1 (#12)	-	liquid	100%	n.p.	Severe	Category 2A	Category I	R36	Irritant	Irritant	Cat II	Swanson and Harbell (2000)
Benchmark-Group 2 (#13)	-	liquid	100%	n.p.	Severe	Category 1	Category I	R41	Irritant	Irritant	Cat II	Swanson and Harbell (2000)
All Purpose Cleaner (#5)	-	liquid	100%	n.p.	Severe						Cat I	Swanson et al. (1995)
All Purpose Cleaner (#7)	-	liquid	100%	n.p.	Severe						Cat I	Swanson et al. (1995)
Bathroom Cleaner (#6)	-	liquid	100%	n.p.	Severe	SCNM	Category III	Nonirritant	Irritant	Irritant	Cat I	Swanson et al. (1995)
Cleaner/Degreaser (#13)	-	liquid	100%	n.p.	Severe						Cat I	Swanson et al. (1995)
Degreaser (#16)	-	liquid	100%	n.p.	Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Swanson et al. (1995)
Floor Cleaner (#10)	-	liquid	100%	n.p.	Severe						Cat II	Swanson et al. (1995)
Floor Cleaner (#2)	-	liquid	100%	n.p.	Nonirritant						Cat III	Swanson et al. (1995)
Floor Stripper (#14)	-	liquid	100%	n.p.	Severe						Cat I	Swanson et al. (1995)
Floor Stripper (#17)	-	liquid	100%	n.p.	Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Swanson et al. (1995)
Floor Stripper (#18)	-	liquid	100%	n.p.	Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Swanson et al. (1995)
General Cleaner (#11)	-	liquid	100%	n.p.	Severe						Cat I	Swanson et al. (1995)
General Cleaner (#12)	-	liquid	100%	n.p.	Severe						Cat I	Swanson et al. (1995)
Glass Cleaner (#19)	-	liquid	100%	n.p.	Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Swanson et al. (1995)
Heavy Duty Cleaner (#15)	-	liquid	100%	n.p.	Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Swanson et al. (1995)
Heavy Duty Cleaner/Degreaser (#9	-	liquid	1	n.p.	Severe						Cat I	Swanson et al. (1995)
Meat Room Degreaser (#3)	-	liquid	1	n.p.	Severe						Cat I	Swanson et al. (1995)
Metal Cleaner (#20)	-	liquid	1	n.p.	Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Swanson et al. (1995)
Pot and Pan Cleaner (#8)	-	liquid	1	n.p.	Nonirritant						Cat III	Swanson et al. (1995)
Toilet Bowl Cleaner (#1)	-	liquid	1	n.p.	Mild	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Swanson et al. (1995)

Comparison of In Vivo and In Vitro Ocular Irritancy Classifications: Sorted by Reference

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	*In Vitro* Consensus Classification Severe ≥ 55.1[1]	*In Vivo* GHS[2,3]	*In Vivo* EPA[4,5]	*In Vivo* EU[6,7]	FHSA-20%[8]	FHSA-67%[9]	*In Vitro* Consensus Classification Severe ≥ 75[1]	Reference
Toilet Bowl Cleaner (#4)	-	liquid	1	n.p.	Mild	Nonirritant	SCNM	Nonirritant	Irritant	Inconclusive	Cat III	Swanson et al. (1995)

Abbreviations: CASRN = Chemical Abstract Services Registry Number; SCNM = study criteria not met; n.p. = not provided

[1] Consensus classification represents the overall BCOP ocular irritancy classification assigned for each chemical in the study based on the majority of ocular irritancy classification calls

[2] GHS = Globally Harmonized System (UN 2007)

[3] Eye Irritant Category 1 = irreversible effects on the eye/serious damage to the eye; Category 2A = reversible effects on the eye/irritating to the eyes; Category 2B = reversible effects on the eye/mildly irritating to the eyes; Nonirritant = not an eye irritant

[4] EPA = U.S. Environmental Protection Agency (EPA 2003a).

[5] Toxicity Category I for the Primary Eye Irritation Study = corrosive, or corneal involvement or irritation not reversible within 21 days; Category II = corneal involvement or irritation clearing in 8-21 days; Category III = corneal involvement or irritation clearing in 1-7 days; Category IV: minimal effects clearing in less than 24 hr

[6] EU = European Union (EU 2001).

[7] Risk phrase R41 = risk of serious damage to the eyes; R36 = irritating to the eyes; nonirritant = not an eye irritant.

[8] FHSA=Federal Hazardous Substance Act (2005). FHSA-20% is based on the proportion of positive animals needed to identify a substance as an irritant using the FHSA sequential testing strategy, where 20% of the animals need to demonstrate a positive response for a substance to be identified as an irritant. A substance tested using 3 to 6 animals would not be labeled if ≤ 1/6 animals were positive based on the FHSA criteria. The substance would be labeled as an irritant if there were ≥1 positive animal in a 3 to 5 animal test or ≥2 positive animals in a 6 animal test.

[9] FHSA=Federal Hazardous Substances Act (2005). FHSA-67% is based on the proportion of positive animals needed to identify a substance as an irritant using the "first test" of the FHSA sequential testing strategy, where 67% of the animals need to demonstrate a positive response for a substance to be identified as an irritant. A substance tested using 3 to 6 animals would not be labeled if ≤ 1/6 animals were positive based on the FHSA criteria. The substance would be labeled as an irritant if there were ≥2/3, 3/4, 4/5, or 4/6 positive animals. If 1/3, 1/4, 2/4, 1/5, 2/5, 3/5, 2/6, or 3/6 animals were positive, further testing would be required.

Annex III-2

BCOP Data Sorted by Substance Name

This page intentionally left blank

Comparison of In Vivo and In Vitro Ocular Irritancy Classifications: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	*In Vitro* Consensus Classification Severe ≥ 55.1[1]	*In Vivo* GHS[2,3]	*In Vivo* EPA[4,5]	*In Vivo* EU[6,7]	FHSA-20%[8]	FHSA-67%[9]	*In Vitro* Consensus Classification Severe ≥ 75[1]	Reference
1-1 (#1)	-	liquid	100%	n.p.	Severe	Category 2A	Category I	R36	Irritant	Irritant	Cat I	Swanson and Harbell (2000)
1-2 (#2)	-	liquid	100%	n.p.	Mild	Category 2A	Category II	R36	Irritant	Irritant	Cat III	Swanson and Harbell (2000)
1-3 (#3)	-	liquid	100%	n.p.	Moderate	Category 2A	Category II	R36	Irritant	Irritant	Cat II	Swanson and Harbell (2000)
1-Naphthalene acetic acid	86-87-3	solid	20%	96	Very Severe	Category 1	Category I	SCNM	Irritant	Irritant	Cat II	Balls et al. (1995)
1-Naphthalene acetic acid, Na salt	61-31-4	solid	20%	95	Very Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Balls et al. (1995)
1-Nitropropane	108-03-2	liquid	100%	n.p.	Mild	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Gautheron et al. (1994)
1-Phenyl-3-pyrazolidone	92-43-3	solid	20%	n.p.	Mild	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat III	Gautheron et al. (1994)
1,2,3-Trichloropropane	96-18-4	liquid	100%	n.p.	Moderate	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat II	Gautheron et al. (1994)
1,2,4-Trimethylbenzene	95-63-6	liquid	100%	n.p.	Mild	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat III	Gautheron et al. (1994)
2-4 (#4)	-	liquid	100%	n.p.	Mild	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Swanson and Harbell (2000)
2-7 (#7)	-	liquid	100%	n.p.	Moderate	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Swanson and Harbell (2000)
2-8 (#8)	-	liquid	100%	n.p.	Moderate	Nonirritant	Category III	Nonirritant	Irritant	Inconclusive	Cat II	Swanson and Harbell (2000)
2-Aminophenol	95-55-6	solid	20%	n.p.	Mild	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Gautheron et al. (1994)
2-Chloro-2,4,4-trimethylpentane	-	liquid	100%	n.p.	Mild	Nonirritant	Category IV	Nonirritant	Irritant	Irritant	Cat III	Bailey et al. (2004)
2-Ethoxyethanol	110-80-5	liquid	100%	n.p.	Severe	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat I	Gautheron et al. (1994)
2-Ethyl-1-hexanol	104-76-7	liquid	100%	99	Moderate	Category 2A	Category II	R36	Irritant	Irritant	Cat II	Balls et al. (1995)
2-Mercaptopyrimidine	1450-85-7	solid	20%	n.p.	Nonirritant	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Gautheron et al. (1994)
2-Methoxyethanol	109-86-4	liquid	100%	n.p.	Severe	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat II	Gautheron et al. (1994)
2,2-Dimethylbutanoic acid	595-37-9	liquid	100%	96	Very Severe	SCNM	Category I	SCNM	Irritant	Irritant	Cat I	Balls et al. (1995)
2,4-Dichloro-5-sulfamoylbenzoic acid	2736-23-4	solid	20%	n.p.	Mild	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat III	Gautheron et al. (1994)

Comparison of In Vivo and In Vitro Ocular Irritancy Classifications: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	*In Vitro* Consensus Classification Severe ≥ 55.1[1]	*In Vivo* GHS[2,3]	*In Vivo* EPA[4,5]	*In Vivo* EU[6,7]	FHSA-20%[8]	FHSA-67%[9]	*In Vitro* Consensus Classification Severe ≥ 75[1]	Reference
2,4-Pentanedione	123-54-6	liquid	100%	n.p.	Severe	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat II	Gautheron et al. (1994)
2,5-Dimethylhexanediol	110-03-2	solid	20%	99.5	Mild	Category 1	Category I	R41	Irritant	Irritant	Cat III	Balls et al. (1995)
2,6-Dichlorobenzoyl chloride	4659-45-4	liquid	100%	99	Mild	Category 2A	Category II	SCNM	Irritant	Irritant	Cat III	Balls et al. (1995)
3-Glycidoxypropyltrimethoxysilane	2530-83-8	liquid	100%	n.p.	Mild	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Gautheron et al. (1994)
4-Carboxybenzaldehyde	619-66-9	solid	20%	95	Severe	Category 2A	Category II	R36	Irritant	Irritant	Cat II	Balls et al. (1995)
4-Carboxybenzaldehyde	619-66-9	solid	20%	95	Moderate	Category 2A	Category II	R36	Irritant	Irritant	Cat II	Southee (1998)
5-Ethylidene-2-norbornene	16219-75-3	liquid	100%	n.p.	Mild	Nonirritant	Category IV	Nonirritant			Cat III	Bailey et al. (2004)
A		n.p.[12]	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
AB		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
AC		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
Acetone	67-64-1	liquid	100%	99	Very Severe	Category 2A	Category II	R36	Irritant	Irritant	Cat I	Balls et al. (1995)
AD		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
AE		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat II	AMCP BRD
AF		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category IV	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD
AG		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Inconclusive	Cat I	AMCP BRD
AH		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	SCNM	SCNM	Cat I	AMCP BRD
AI		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
AJ		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
AK		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
AL		n.p.	n.p.	n.p.	Cat I	Category 2A	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
Alkyl phosphoric acid ester/amine	-	liquid	100%	n.p.	Severe	Category 1	SCNM	R41	Irritant	Irritant	Cat I	Bailey et al. (2004)

Comparison of In Vivo and In Vitro Ocular Irritancy Classifications: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	*In Vitro* Consensus Classification Severe ≥ 55.1[1]	*In Vivo* GHS[2,3]	*In Vivo* EPA[4,5]	*In Vivo* EU[6,7]	FHSA-20%[8]	FHSA-67%[9]	*In Vitro* Consensus Classification Severe ≥ 75[1]	Reference
All Purpose Cleaner (#5)	-	liquid	100%	n.p.	Severe						Cat I	Swanson et al. (1995)
All Purpose Cleaner (#7)	-	liquid	100%	n.p.	Severe						Cat I	Swanson et al. (1995)
Allyl alcohol	107-18-6	liquid	100%	n.p.	Severe	Category 2A	Category III	R36	Irritant	Irritant	Cat I	Gautheron et al. (1994)
Aluminum hydroxide	21645-51-2	solid	20%	n.p.	Mild	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat III	Gautheron et al. (1994)
AM		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
Ammonium nitrate	6484-52-2	solid	20%	>99.9	Mild	Category 2B	Category III	R36	Irritant	Irritant	Cat III	Balls et al. (1995)
Ammonium nitrate	6484-52-2	n.p.	100%	n.p.	Mild	Category 2B	Category III	R36	Irritant	Irritant	Mild	Southee (1998)
AN		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Not labeled	Not labeled	Cat I	AMCP BRD
Anthracene	120-12-7	solid	20%	n.p.	Nonirritant	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Gautheron et al. (1994)
AO		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
AP		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
AQ		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
AR		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
Aromatic hydrocarbon #1	-	liquid	100%	n.p.	Nonirritant	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Bailey et al. (2004)
Aromatic hydrocarbon #2	-	liquid	100%	n.p.	Mild	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Bailey et al. (2004)
Aryl phosponates	-	liquid	100%	n.p.	Moderate	Category 2B	SCNM	SCNM	Irritant	Irritant	Cat II	Bailey et al. (2004)
AS		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
AT		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
AU		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
AV		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	SCNM	SCNM	Cat I	AMCP BRD
AW		n.p.	n.p.	n.p.	Cat II	Category 1	Category I	n.p.	Irritant	Irritant	Cat II	AMCP BRD

Comparison of In Vivo and In Vitro Ocular Irritancy Classifications: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	*In Vitro* Consensus Classification Severe ≥ 55.1[1]	*In Vivo* GHS[2,3]	*In Vivo* EPA[4,5]	*In Vivo* EU[6,7]	FHSA-20%[8]	FHSA-67%[9]	*In Vitro* Consensus Classification Severe ≥ 75[1]	Reference
AX		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
AY		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
B		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
Bathroom Cleaner (#6)	-	liquid	100%	n.p.	Severe	SCNM	Category III	Nonirritant	Irritant	Irritant	Cat I	Swanson et al. (1995)
BB		n.p.	n.p.	n.p.	Cat III	SCNM	Category IV	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD
BD		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category III	n.p.	Irritant	Irritant	Cat III	AMCP BRD
BE		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category III	n.p.	Irritant	Inconclusive	Cat III	AMCP BRD
Benchmark-Group 1 (#12)	-	liquid	100%	n.p.	Severe	Category 2A	Category I	R36	Irritant	Irritant	Cat II	Swanson and Harbell (2000)
Benchmark-Group 2 (#13)	-	liquid	100%	n.p.	Severe	Category 1	Category I	R41	Irritant	Irritant	Cat II	Swanson and Harbell (2000)
Benzalkonium chloride (1 %)	8001-54-5	liquid	1%	98	Very Severe	Category 1	Category II	R41	Irritant	Irritant	Cat I	Balls et al. (1995)
Benzalkonium chloride (10%)	8001-54-5	liquid	10%	98	Very Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Balls et al. (1995)
Benzalkonium chloride (100%)	8001-54-5	liquid	10%	n.p.	Very Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Southee (1998)
Benzalkonium chloride (5%)	8001-54-5	liquid	5%	98	Very Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Balls et al. (1995)
Benzethonium chloride	121-54-0	surfactant	10%	n.p.	Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Gautheron et al. (1994)
Benzoyl-L-tartaric acid	2743-38-6	solid	20%		Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Balls et al. (1995)
Betaine monohydrate	590-47-6	solid	20%	n.p.	Mild	Nonirritant	Category III	Nonirritant	Irritant	Inconclusive	Cat III	Gautheron et al. (1994)
BF		n.p.	n.p.	n.p.	Cat I	Category 2A	Category III	n.p.	Irritant	Irritant	Cat II	AMCP BRD
BJ		n.p.	n.p.	n.p.	Cat I	Nonirritant	Category III	n.p.	Irritant	Irritant	Cat I	AMCP BRD
BJ		n.p.	n.p.	n.p.	Cat II	Nonirritant	Category III	n.p.	Irritant	Irritant	Cat II	AMCP BRD
BK		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category III	n.p.	Irritant	Irritant	Cat III	AMCP BRD
BL		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category IV	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD

Comparison of In Vivo and In Vitro Ocular Irritancy Classifications: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	*In Vitro* Consensus Classification Severe ≥ 55.1[1]	*In Vivo* GHS[2,3]	*In Vivo* EPA[4,5]	*In Vivo* EU[6,7]	FHSA-20%[8]	FHSA-67%[9]	*In Vitro* Consensus Classification Severe ≥ 75[1]	Reference
BM		n.p.	n.p.	n.p.	Cat II	Nonirritant	Category IV	n.p.	Not labeled	Not labeled	Cat II	AMCP BRD
BN		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category IV	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD
BP		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category IV	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD
BQ		n.p.	n.p.	n.p.	Cat II	Nonirritant	Category IV	n.p.	Not labeled	Not labeled	Cat II	AMCP BRD
BRIJ-35	9002-92-0	surfactant	10%	n.p.	Nonirritant	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Gautheron et al. (1994)
Butyl acetate	123-86-4	liquid	100%	99	Moderate	Nonirritant	Category III	Nonirritant	Irritant	Inconclusive	Cat II	Balls et al. (1995)
Butyl cellusolve	111-76-2	liquid	100%	n.p.	Very Severe	Category 1	Category II	R41	Irritant	Irritant	Cat I	Southee (1998)
Butyrolactone	96-48-0	liquid	100%	n.p.	Moderate	Category 2A	Category II	R36	Irritant	Irritant	Cat II	Gautheron et al. (1994)
C		n.p.	n.p.	n.p.	Cat II	Category 1	Category I	n.p.	Irritant	Irritant	Cat II	AMCP BRD
Captan 90 concentrate	133-06-2	solid	20%	90	Moderate	Category 1	Category I	R41	Irritant	Irritant	Cat II	Balls et al. (1995)
Carboxylic acid amides	-	solid	100%	n.p.	Moderate	Category 1	Category I	R41	Irritant	Irritant	Cat II	Bailey et al. (2004)
Cetylpyridinium bromide (0.1%)	140-72-7	liquid	0.10%	98	Mild	Nonirritant	Category III	Nonirritant	Irritant	Inconclusive	Cat III	Balls et al. (1995)
Cetylpyridinium bromide (10%)	140-72-7	liquid	10%	98	Very Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Balls et al. (1995)
Cetylpyridinium bromide (6%)	140-72-7	liquid	6%	98	Severe	Category 1	SCNM	R41	Irritant	Irritant	Cat II	Balls et al. (1995)
CG		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category IV	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD
CH		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category III	n.p.	Irritant	Inconclusive	Cat III	AMCP BRD
Chlorhexidine	55-56-1	solid	20%	n.p.	Very Severe	Category 1	SCNM	SCNM	Irritant	Irritant	Cat I	Balls et al. (1995)
Clarified slurry oil	-	liquid	100%	n.p.	Mild	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Bailey et al. (2004)
Cleaner/Degreaser (#13)	-	liquid	100%	n.p.	Severe						Cat I	Swanson et al. (1995)
Cutting fluid (conc.) #1	-	liquid	100%	n.p.	Mild	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Bailey et al. (2004)
Cutting fluid (conc.) #2	-	liquid	100%	n.p.	Mild	Nonirritant	Category III	Nonirritant	Irritant	Inconclusive	Cat III	Bailey et al. (2004)

Comparison of In Vivo and In Vitro Ocular Irritancy Classifications: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	*In Vitro* Consensus Classification Severe ≥ 55.1[1]	*In Vivo* GHS[2,3]	*In Vivo* EPA[4,5]	*In Vivo* EU[6,7]	FHSA-20%[8]	FHSA-67%[9]	*In Vitro* Consensus Classification Severe ≥ 75[1]	Reference
Cyclohexanol	108-93-0	liquid	100%	97	Moderate	Category 1	Category I	R41	Irritant	Irritant	Cat II	Balls et al. (1995)
Cyclohexanone	108-94-1	liquid	100%	n.p.	Severe	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat I	Gautheron et al. (1994)
D		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
Degreaser (#16)	-	liquid	100%	n.p.	Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Swanson et al. (1995)
Deoxycholic acid, sodium salt	302-95-4	surfactant	10%	n.p.	Severe	Category 2A	Category II	R36	Irritant	Irritant	Cat I	Gautheron et al. (1994)
Diacetone alcohol	123-42-2	liquid	100%	n.p.	Moderate	SCNM	SCNM	SCNM	Irritant	Irritant	Cat II	Gautheron et al. (1994)
Dibenzoyl-L-tartaric acid	2743-38-6	solid	20%	n.p.	Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Gautheron et al. (1994)
Dibenzyl phosphate	1623-08-1	solid	20%	99	Very Severe	Category 2A	Category II	R36	Irritant	Irritant	Cat I	Balls et al. (1995)
Dimethyl sulfoxide	67-68-5	liquid	100%	n.p.	Mild	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat III	Gautheron et al. (1994)
Dimethylbiguanide	657-24-9	solid	20%	n.p.	Nonirritant	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat III	Gautheron et al. (1994)
DL-Glutamic acid	19285-83-7	solid	20%	n.p.	Nonirritant	SCNM	SCNM	SCNM	Irritant	Inconclusive	Cat III	Gautheron et al. (1994)
E		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	SCNM	SCNM	Cat I	AMCP BRD
EDTA, di-potassium salt	25102-12-9	solid	20%	n.p.	Nonirritant	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat III	Gautheron et al. (1994)
EF		n.p.	n.p.	n.p.	Cat I	Category 2A	Category II	n.p.	Irritant	Irritant	Cat I	AMCP BRD
EG		n.p.	n.p.	n.p.	Cat I	Category 2A	Category II	n.p.	Irritant	Irritant	Cat II	AMCP BRD
Ethanol	64-17-5	liquid	100%	n.p.	Severe	Category 2A	Category III	Nonirritant	Irritant	Irritant	Cat I	Balls et al. (1995)
Ethanol	64-17-5	liquid	100%	n.p.	Severe	SCNM	Category II	Nonirritant	Irritant	Irritant	Cat I	Gautheron et al. (1994)
Ethanol	64-17-5	liquid	100%	n.p.	Moderate	Category 2A	Category III	Nonirritant	Irritant	Irritant	Cat II	Southee (1998)
Ethyl acetate	141-78-6	liquid	100%	99	Moderate	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat II	Balls et al. (1995)
Ethyl acetoacetate	141-97-9	liquid	100%	n.p.	Moderate	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat II	Gautheron et al. (1994)
Ethyl trimethyl acetate	3938-95-2	liquid	100%	99	Mild	Nonirritant	Category III	Nonirritant	Not labeled	Not labeled	Cat III	Balls et al. (1995)

Comparison of In Vivo and In Vitro Ocular Irritancy Classifications: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	*In Vitro* Consensus Classification Severe ≥ 55.1[1]	*In Vivo* GHS[2,3]	*In Vivo* EPA[4,5]	*In Vivo* EU[6,7]	FHSA-20%[8]	FHSA-67%[9]	*In Vitro* Consensus Classification Severe ≥ 75[1]	Reference
Ethyl-2-methylacetoacetate	609-14-3	liquid	100%	97	Mild	Category 2B	Category III	Nonirritant	Irritant	Irritant	Cat III	Balls et al. (1995)
Ethylhexyl acid phosphate ester	-	liquid	100%	n.p.	Severe	Category 1	SCNM	R41	Irritant	Irritant	Cat I	Bailey et al. (2004)
F		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
Floor Cleaner (#10)	-	liquid	100%	n.p.	Severe						Cat II	Swanson et al. (1995)
Floor Cleaner (#2)	-	liquid	100%	n.p.	Nonirritant						Cat III	Swanson et al. (1995)
Floor Stripper (#14)	-	liquid	100%	n.p.	Severe						Cat I	Swanson et al. (1995)
Floor Stripper (#17)	-	liquid	100%	n.p.	Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Swanson et al. (1995)
Floor Stripper (#18)	-	liquid	100%	n.p.	Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Swanson et al. (1995)
Fomesafen	72128-02-0	solid	20%	97.5	Severe	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat II	Balls et al. (1995)
Furan	110-00-9	liquid	100%	n.p.	Severe	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat II	Gautheron et al. (1994)
G		n.p.	n.p.	n.p.	Cat I	Category 1	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
gamma-Butyrolactone	96-48-0	liquid	100%	>99	Severe	Category 2A	Category II	R36	Irritant	Irritant	Cat II	Balls et al. (1995)
General Cleaner (#11)	-	liquid	100%	n.p.	Severe						Cat I	Swanson et al. (1995)
General Cleaner (#12)	-	liquid	100%	n.p.	Severe						Cat I	Swanson et al. (1995)
Glass Cleaner (#19)	-	liquid	100%	n.p.	Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Swanson et al. (1995)
Gluconolactone	90-80-2	solid	20%	n.p.	Severe	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat I	Gautheron et al. (1994)
Glycerol	56-81-5	liquid	100%	>99.5	Nonirritant	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Balls et al. (1995)
Glycerol	56-81-5	liquid	100%	>99.5	Nonirritant	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Nonirritant	Southee (1998)
H		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category II	n.p.	Irritant	Irritant	Cat III	AMCP BRD
Heavy Duty Cleaner (#15)	-	liquid	100%	n.p.	Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Swanson et al. (1995)
Heavy Duty Cleaner/Degreaser (#9)	-	liquid	100%	n.p.	Severe						Cat I	Swanson et al. (1995)
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	Moderate	Category 1	Category I	R41	Irritant	Irritant	Cat II	Gautheron et al. (1994)

Comparison of In Vivo and In Vitro Ocular Irritancy Classifications: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	*In Vitro* Consensus Classification Severe ≥ 55.1[1]	*In Vivo* GHS[2,3]	*In Vivo* EPA[4,5]	*In Vivo* EU[6,7]	FHSA-20%[8]	FHSA-67%[9]	*In Vitro* Consensus Classification Severe ≥ 75[1]	Reference
Hexadecyltrimethylammonium bromide	57-09-0	surfactant	10%	n.p.	Moderate	Category 1	Category I	R41	Irritant	Irritant	Cat II	Southee (1998)
Hexane	110-54-3	liquid	100%	n.p.	Nonirritant	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Gautheron et al. (1994)
I		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category III	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD
Imidazole	288-32-4	solid	20%	99	Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Balls et al. (1995)
Imidazole	288-32-4	solid	20%	n.p.	Severe	Category 1	Category I	R41	Irritant	Irritant		Gautheron et al. (1994)
Imidazole	288-32-4	solid	20%	n.p.	Very Severe	Category 1	Category I	R41	Irritant	Irritant	Very Severe	Southee (1998)
Iminodibenzyl	494-19-9	solid	20%	n.p.	Nonirritant	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat III	Gautheron et al. (1994)
Isobutanol	78-83-1	liquid	100%	99.9	Moderate	Category 2A	Category II	R36	Irritant	Irritant	Cat II	Balls et al. (1995)
Isopropanol	67-63-0	liquid	100%	99.9	Severe	Category 2A	Category III	SCNM	Irritant	Irritant	Cat II	Balls et al. (1995)
J		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category III	n.p.	Irritant	Irritant	Cat III	AMCP BRD
K		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category IV	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD
L		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category III	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD
L-Aspartic acid	70-47-3	solid	20%	100	Nonirritant	SCNM	SCNM	SCNM	Irritant	Irritant	Cat III	Balls et al. (1995)
Laurylsulfobetaine	14933-08-5	surfactant	10%	n.p.	Severe	SCNM	SCNM	SCNM	Irritant	Irritant	Cat I	Gautheron et al. (1994)
M		n.p.	n.p.	n.p.	Cat I	Nonirritant	Category III	n.p.	Irritant	Irritant	Cat II	AMCP BRD
Magnesium carbonate	56378-72-4	solid	20%	n.p.	Nonirritant	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat III	Gautheron et al. (1994)
Maneb	12427-38-2	solid	20%	90	Mild	SCNM	Category III	SCNM	Irritant	Irritant	Cat II	Balls et al. (1995)
Meat Room Degreaser (#3)	-	liquid	100%	n.p.	Severe						Cat I	Swanson et al. (1995)
Metal Cleaner (#20)	-	liquid	100%	n.p.	Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Swanson et al. (1995)
Methanol	67-56-1	liquid	100%	n.p.	Severe	Nonirritant	Category II	Nonirritant	Irritant	Irritant	Cat I	Gautheron et al. (1994)
Methyl acetate	79-20-9	liquid	100%	98	Moderate	Category 2A	Category II	R36	Irritant	Irritant	Cat II	Balls et al. (1995)

Comparison of In Vivo and In Vitro Ocular Irritancy Classifications: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	*In Vitro* Consensus Classification Severe ≥ 55.1[1]	*In Vivo* GHS[2,3]	*In Vivo* EPA[4,5]	*In Vivo* EU[6,7]	FHSA-20%[8]	FHSA-67%[9]	*In Vitro* Consensus Classification Severe ≥ 75[1]	Reference
Methyl cyanoacetate	105-34-0	liquid	100%	99	Mild	Category 2A	Category II	R36	Irritant	Irritant	Cat III	Balls et al. (1995)
Methyl cyclopentadiene dimer	-	liquid	100%	n.p.	Nonirritant	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Bailey et al. (2004)
Methyl ethyl ketone	78-93-3	liquid	100%	99	Severe	Category 2A	Category III	R36	Irritant	Irritant	Cat II	Balls et al. (1995)
Methyl ethyl ketone	78-93-3	liquid	100%	99	Severe	Category 2B	Category III	R36	Irritant	Irritant	Cat II	Southee (1998)
Methyl isobutyl ketone	108-10-1	liquid	100%	98	Mild	Nonirritant	Category III	Nonirritant	Irritant	Inconclusive	Cat III	Balls et al. (1995)
Methylcyclopentane	96-37-7	liquid	100%	>99	Nonirritant	Nonirritant	Category III	Nonirritant	Not labeled	Not labeled	Cat III	Balls et al. (1995)
Methylisobutyl ketone	108-10-1	liquid	100%	n.p.	Mild	Nonirritant	Category III	Nonirritant	Irritant	Inconclusive	Cat III	Gautheron et al. (1994)
MYRJ-45	-	surfactant	10%	n.p.	Nonirritant	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Gautheron et al. (1994)
N		n.p.	n.p.	n.p.	Cat I	Nonirritant	Category III	n.p.	Irritant	Inconclusive	Cat I	AMCP BRD
n-Hexanol	111-27-3	liquid	100%	98	Severe/Very Severe	Category 2A	Category II	R36	Irritant	Irritant	Cat II	Balls et al. (1995)
N-Lauroylsarcosine, sodium salt	7631-98-3	surfactant	10%	n.p.	Moderate	Category 2B	Category III	Nonirritant	Irritant	Irritant	Cat II	Gautheron et al. (1994)
n-Octanol	111-87-5	liquid	100%	>99	Moderate	Category 2B	Category III	R36	Irritant	Irritant	Cat II	Balls et al. (1995)
O		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category IV	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD
Octanol	111-87-5	liquid	100%	n.p.	Moderate	Category 2B	Category III	R36	Irritant	Irritant	Cat II	Gautheron et al. (1994)
P		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category IV	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD
Parafluoraniline	371-40-4	liquid	100%	99	Moderate	SCNM	SCNM	SCNM	Irritant	Irritant	Cat II	Balls et al. (1995)
Parafluoraniline	371-40-4	liquid	100%	99	Moderate	SCNM	SCNM	SCNM	Irritant	Irritant	Cat II	Southee (1998)
Petroleum ether	8032-32-4	liquid	100%	n.p.	Mild	Nonirritant	Category III	Nonirritant	Irritant	Inconclusive	Cat III	Gautheron et al. (1994)
Petroleum wax	-	solid	100%	n.p.	Nonirritant	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Bailey et al. (2004)
Phenylbutazone	50-33-9	solid	20%	n.p.	Nonirritant	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Gautheron et al. (1994)
Polyalkenylsuccinate ester/amine s	-	liquid	100%	n.p.	Nonirritant	SCNM	Category III	SCNM	Irritant	Irritant	Cat III	Bailey et al. (2004)

Comparison of In Vivo and In Vitro Ocular Irritancy Classifications: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	*In Vitro* Consensus Classification Severe ≥ 55.1[1]	*In Vivo* GHS[2,3]	*In Vivo* EPA[4,5]	*In Vivo* EU[6,7]	FHSA-20%[8]	FHSA-67%[9]	*In Vitro* Consensus Classification Severe ≥ 75[1]	Reference
Polyethylene glycol 400	25322-68-3	liquid	100%	n.p.	Nonirritant	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Balls et al. (1995)
Pot and Pan Cleaner (#8)	-	liquid	100%	n.p.	Nonirritant						Cat III	Swanson et al. (1995)
Potassium cyanate	590-28-3	solid	20%	97	Mild	SCNM	SCNM	SCNM	Irritant	Irritant	Cat III	Balls et al. (1995)
Process oil	-	liquid	100%	n.p.	Nonirritant	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Bailey et al. (2004)
Promethazine hydrochloride	58-33-3	solid	20%	98	Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Balls et al. (1995)
Promethazine hydrochloride	58-33-3	solid	20%	n.p.	Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Gautheron et al. (1994)
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	n.p.	Mild	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat III	Gautheron et al. (1994)
Propyl-4-hydroxybenzoate	94-13-3	solid	20%	100	Mild	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat III	Southee (1998)
Pyridine	110-86-1	liquid	100%	>99.9	Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Balls et al. (1995)
Pyridine	110-86-1	liquid	100%	n.p.	Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Gautheron et al. (1994)
Q		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category IV	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD
Quinacrine	69-05-6	solid	20%	n.p.	Mild	Category 1	Category I	R41	Irritant	Irritant	Cat III	Balls et al. (1995)
Quinacrine	69-05-6	solid	20%	n.p.	Moderate	Category 1	Category I	R41	Irritant	Irritant	Cat III	Gautheron et al. (1994)
R		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category IV	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD
S		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category IV	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD
Sodium hydroxide (1%)	1310-73-2	liquid	1%	reagent grade	Very Severe	Category 2B	Category III	R36	Irritant	Irritant	Cat I	Balls et al. (1995)
Sodium hydroxide (10%)	1310-73-2	liquid	10%	reagent grade	Severe	Category 1	Category I	R41	SCNM	SCNM	Cat I	Balls et al. (1995)
Sodium hydroxide (10%)	1310-73-2	liquid	10%	n.p.	Very Severe	Category 1	Category I	R41	SCNM	SCNM	Cat I	Southee (1998)
Sodium lauryl sulfate (15 %)	151-21-3	liquid	15%	98	Mild	Category 1	Category I	R36	Irritant	Irritant	Cat II	Balls et al. (1995)
Sodium lauryl sulfate (15%)	151-21-3	liquid	15%	98	Mild	Category 1	Category I	SCNM	Irritant	Irritant	Cat III	Southee (1998)
Sodium lauryl sulfate (3 %)	151-21-3	liquid	3%	98	Moderate	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat II	Balls et al. (1995)

Comparison of In Vivo and In Vitro Ocular Irritancy Classifications: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	*In Vitro* Consensus Classification Severe ≥ 55.1[1]	*In Vivo* GHS[2,3]	*In Vivo* EPA[4,5]	*In Vivo* EU[6,7]	FHSA-20%[8]	FHSA-67%[9]	*In Vitro* Consensus Classification Severe ≥ 75[1]	Reference
Sodium oxalate	62-76-0	solid	20%	>99	Mild	Category 1	Category I	R41	Irritant	Irritant	Cat III	Balls et al. (1995)
Sodium oxalate	62-76-0	solid	20%	n.p.	Mild	Category 1	Category I	R41	Irritant	Irritant		Gautheron et al. (1994)
Sodium oxalate	62-76-0	solid	20%	99	Nonirritant	Category 1	Category I	R41	Irritant	Irritant	Cat III	Southee (1998)
Sodium perborate	10486-00-7	solid	20%	98.6	Very Severe	Category 1	Category I	R41	Irritant	Irritant	Cat I	Balls et al. (1995)
T		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category IV	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	97	Mild	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat III	Balls et al. (1995)
Tetraaminopyrimidine sulfate	5392-28-9	solid	20%	n.p.	Mild	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat III	Gautheron et al. (1994)
Thiadiazole alkyl derivative	-	liquid	100%	n.p.	Moderate	SCNM	Category III	SCNM	Irritant	Irritant	Cat III	Bailey et al. (2004)
Thiourea	62-56-6	solid	20%	>99	Severe	SCNM	SCNM	SCNM	Irritant	Irritant	Cat I	Balls et al. (1995)
Thiourea	62-56-6	solid	20%	n.p.	Severe	SCNM	SCNM	SCNM	Irritant	Irritant	Cat I	Gautheron et al. (1994)
Toilet Bowl Cleaner (#1)	-	liquid	100%	n.p.	Mild	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Swanson et al. (1995)
Toilet Bowl Cleaner (#4)	-	liquid	100%	n.p.	Mild	Nonirritant	SCNM	Nonirritant	Irritant	Inconclusive	Cat III	Swanson et al. (1995)
Toluene	108-88-3	liquid	100%	99	Moderate	Nonirritant	Category III	Nonirritant	Irritant	Irritant	Cat II	Balls et al. (1995)
Trichloroacetic acid (3%)	76-03-9	liquid	3%	reagent grade	Severe/Very Severe	Nonirritant	Category III	Nonirritant	Irritant	Inconclusive	Cat I	Balls et al. (1995)
Trichloroacetic acid (30%)	76-03-9	liquid	30%	reagent grade	Very Severe	Category 1	Category I	R41	SCNM	SCNM	Cat I	Balls et al. (1995)
Triethanolamine	102-71-6	liquid	100%	n.p.	Nonirritant	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Gautheron et al. (1994)
Triton X-100 (10%)	9002-93-1	liquid	10%	98	Severe/Very Severe	Category 1	Category II	R41	Irritant	Irritant	Cat I	Balls et al. (1995)
Triton X-100 (5 %)	9002-93-1	liquid	5%	98	Severe	Category 2A	Category III	R36	Irritant	Irritant	Cat I	Balls et al. (1995)
Triton X-100 (5%)	9002-93-1	liquid	5%	98	Mild	Category 2B	Category III	R36	Irritant	Irritant	Cat III	Southee (1998)
Triton X-155	9010-44-0	surfactant	10%	n.p.	Nonirritant	Nonirritant	Category IV	Nonirritant	Not labeled	Not labeled	Cat III	Gautheron et al. (1994)
Tween 20	9005-64-5	liquid	n.p.	98	Nonirritant	Nonirritant	Category III	Nonirritant	Irritant	Inconclusive	Cat III	Balls et al. (1995)

Comparison of In Vivo and In Vitro Ocular Irritancy Classifications: Sorted by Substance

Substance	CASRN	Form Tested	Concentration Tested	Purity (%)	*In Vitro* Consensus Classification Severe ≥ 55.1[1]	*In Vivo* GHS[2,3]	*In Vivo* EPA[4,5]	*In Vivo* EU[6,7]	FHSA-20%[8]	FHSA-67%[9]	*In Vitro* Consensus Classification Severe ≥ 75[1]	Reference
Tween 20	9005-64-5	liquid	100%	98	Nonirritant	Nonirritant	Category III	Nonirritant	Irritant	Inconclusive	Nonirritant	Southee (1998)
U		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category IV	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD
V		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category IV	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD
W		n.p.	n.p.	n.p.	Cat III	Nonirritant	Category IV	n.p.	Not labeled	Not labeled	Cat III	AMCP BRD
X		n.p.	n.p.	n.p.	Cat I	Category 2A	Category I	n.p.	Irritant	Irritant	Cat I	AMCP BRD
Y		n.p.	n.p.	n.p.	Cat I	Category 2A	Category II	n.p.	Irritant	Irritant	Cat II	AMCP BRD
Z		n.p.	n.p.	n.p.	Cat II	Category 2A	Category II	n.p.	Irritant	Irritant	Cat II	AMCP BRD

Abbreviations: CASRN = Chemical Abstract Services Registry Number; SCNM = study criteria not met; n.p. = not provided

[1] Consensus classification represents the overall BCOP ocular irritancy classification assigned for each chemical in the study based on the majority of ocular irritancy classification calls

[2] GHS=Globally Harmonized System (UN 2007)

[3] Eye Irritant Category 1 = irreversible effects on the eye/serious damage to the eye; Category 2A = reversible effects on the eye/irritating to the eyes; Category 2B = reversible effects on the eye/mildly irritating to the eyes; Nonirritant = not an eye irritant

[4] EPA = U.S. Environmental Protection Agency (EPA 2003a).

[5] Toxicity Category I for the Primary Eye Irritation Study = corrosive, or corneal involvement or irritation not reversible within 21 days; Category II = corneal involvement or irritation clearing in 8-21 days; Category III = corneal involvement or irritation clearing in 1-7 days; Category IV: minimal effects clearing in less than 24 hr

[6] EU=European Union (EU 2001).

[7] Risk phrase R41 = risk of serious damage to the eyes; R36 = irritating to the eyes; nonirritant = not an eye irritant.

[8] FHSA=Federal Hazardous Substance Act (2005). FHSA-20% is based on the proportion of positive animals needed to identify a substance as an irritant using the FHSA sequential testing strategy, where 20% of the animals need to demonstrate a positive response for a substance to be identified as an irritant. A substance tested using 3 to 6 animals would not be labeled if ≤ 1/6 animals were positive based on the FHSA criteria. The substance would be labeled as an irritant if there were ≥1 positive animal in a 3 to 5 animal test or ≥2 positive animals in a 6 animal test.

[9] FHSA=Federal Hazardous Substances Act (2005). FHSA-67% is based on the proportion of positive animals needed to identify a substance as an irritant using the "first test" of the FHSA sequential testing strategy, where 67% of the animals need to demonstrate a positive response for a substance to be identified as an irritant. A substance tested using 3 to 6 animals would not be labeled if ≤ 1/6 animals were positive based on the FHSA criteria. The substance would be labeled as an irritant if there were ≥2/3, 3/4, 4/5, or 4/6 positive animals. If 1/3, 1/4, 2/4, 1/5, 2/5, 3/5, 2/6, or 3/6 animals were positive, further testing would be required.

Appendix D

Background Review Document of Existing Methods for Eye Irritation Testing: Silicon Microphysiometer and Cytosensor Microphysiometer

Only the body of the document is included here. The full document, including all appendices, is available electronically on the enclosed CD-ROM or at:
http://iccvam.niehs.nih.gov/methods/ocutox/CM/ECVAM-CMBRD-Aug08redact.pdf
and
http://iccvam.niehs.nih.gov/methods/ocutox/CM/CMBRD-AnnexesAug08redact.pdf

The document is also available on request from NICEATM:

NICEATM
National Institute of Environmental Health Sciences
P.O. Box 12233, MD K2-16
Research Triangle Park, NC 27709 USA
Telephone: 919-541-2384 Fax: 919-541-0947
E-mail: niceatm@niehs.nih.gov

This page intentionally left blank

Background Review Document of Existing Methods for Eye Irritation Testing:

Silicon Microphysiometer and Cytosensor Microphysiometer

Contract No. CCR.IHCP.C431305.X0

Prepared for:

European Commission
Directorate-General JRC
Joint Research Centre
Institute for Health and Consumer Protection

Prepared by:

Institute for In Vitro Sciences, Inc.
30 W. Watkins Mill Road, Suite 100
Gaithersburg, MD 20878
USA

Authors:

Rodger D. Curren, Ph.D.
Angela Sizemore, B.S.
Jennifer Nash, M.S.
Greg Mun, B.S
Amanda Ulrey, RQAP-GLP
John W. Harbell, Ph.D.

Final Revision

20 August 2008

TABLE OF CONTENTS

List of Tables v

List of Figures xvi

List of Annexes xxii

List of Abbreviations xxiv

Acknowledgements xxv

1. Data collection 1

1.1 Description of the methods used to collect data, including literature search or other sources, and number of studies collected 1
1.1.1 Studies identified outside of database searches 5
1.2 Brief description of data collected on overall study management 6

2. Test Definition (Module 1) 10

2.1 Rationale for the proposed test method 10
2.1.1 Intended uses / purpose 10
2.1.2 Regulatory rational and applicability 10
2.1.3 Scientific basis for the test 10
2.1.4 Similarities and differences of modes of action in the test method and the reference species 15
2.2 Test method protocols 17
2.2.1 Description of protocol components and rationale for differences 20
2.2.1.1 Development of Conversion Algorithm between SM and CM 23
2.2.2 Proposed critical components of the protocol that impact on reproducibility and/or predictive capacity of the assay 25
2.2.3 List of studies with similar protocols 27
2.2.4 Known applicability and limitations of the assay 27
2.2.5 Proposed prediction models 28

3. Within-laboratory reproducibility (Module 2) 31

3.1 Within-laboratory reproducibility for studies where raw data are available 31
3.2 Compilation of results 33

3.2.1 Statistical approaches used: description & rationale for the approach used 33
3.2.2 Results and discussion 33
3.2.2.1 Company # 1 study 33
3.2.2.2 Company # 2 study 34
3.2.2.3 CTFA Phase III study 35
3.2.2.4 EC/HO study 38
3.2.2.5 COLIPA study 40
3.2.2.6 SM and CM comparison study 46
3.2.2.7 Materials common to the EC/HO and the COLIPA study (Type II reproducibility) 47
3.2.2.8 Historic positive control data from Company # 3. 50
3.2.3 Additional information/evaluation – Operator Variability 51
3.3 Additional studies where raw data are not available: attempt to combine the data using weigh-of-evidence approaches 51
3.3.1 Protocols and long term intralaboratory reproducibility 51
3.3.2 Test materials 52
3.3.3 Classifications 52

4. Transferability (Module 3) 54

4.1 Brief description of study results on transferability and availability of Standardised Operating Procedures (SOPs) 54
4.2 Facilities and major fixed equipment needed 58
4.3 Required level of training, expertise, and demonstrated proficiency needed 59

5. Between-laboratory reproducibility (Module 4) 61

5.1 Relevant results and information for studies where raw data is available 61
5.2 Discussions, e.g., were sources of variability taken into consideration 63
5.2.1 Bagley, Bruner et al. 1992 study 63
5.2.2 EC/HO study 65
5.2.2.1 Reproducibility of "testable" decision for EC/HO study 66
5.2.2.2 Reproducibility of MRD_{50} values for the EC/HO study 67
5.2.2.3 Reproducibility of predicted hazard classifications for the EC/HO study 71
5.2.3 COLIPA study 76
5.2.3.1 Reproducibility of "testable" decision for COLIPA study 77
5.2.3.2 Reproducibility of MRD_{50} values for the COLIPA study 77
5.2.3.3 Reproducibility of predicted hazard classifications for the COLIPA study 81
5.2.4 Analysis of materials common to both the EC/HO and COLIPA studies 84

5.2.5 Analysis of materials common to both the CTFA and COLIPA studies 91
5.3 Compilation of results, statistical approaches used: description & rationale for the approach used to determine between-laboratory reproducibility 93
5.4 Additional studies where raw data are not available: attempt to combine the data using weight-of-evidence approaches 93

6. Predictive Capacity (Module 5) 94

6.1 Studies with available raw data 97
6.1.1 Relevant information for each study where raw data are available 100
6.1.2 *In vivo* reference data used to assess the performance of the alternative method 101
6.1.3 Brief description of the studies with available raw data 103
6.1.3.1 Analysis of the EC/HO study (Balls, Botham et al. 1995) 103
6.1.3.2 Analysis of the CTFA Phase III study (Gettings, Lordo et al. 1996) 115
6.1.3.3 Analysis of the COLIPA study (Brantom, Bruner et al. 1997) 122
6.1.3.4 Analysis of the combined CTFA Phase III, EC/HO, and COLIPA data 134
6.1.3.4.1 Analysis of the combined CTFA, EC/HO, and COLIPA data with EU category 139
6.1.3.4.2 Analysis of the combined CTFA, EC/HO, and COLIPA data with GHS category 144
6.1.3.4.3 Analysis of the combined CTFA, EC/HO, and COLIPA data with EPA category 150
6.1.3.5 Analysis of the Company # 1 unpublished data 160
6.1.4 Compilation of data on predictive capacity of the test method 170
6.1.4.1 Description & rational for the prediction model(s) applied and statistical approaches used 170
6.1.4.2 Description of performance compared to reference and eventually, to the human situation for each study 173
6.1.4.3 Discussions 175
6.2 Additional studies where raw data are not available 176
6.2.1 Relevant information for each study where raw data are not available 177
6.2.2 Relevant information for *in vivo* study where raw data are available 178
6.2.3. Brief description of the studies without available raw data 179
6.2.3.1 Company # 1 study (Bruner, Miller et al., 1991) 179

6.2.3.2 Multiple lab study (Bagley, Brunner et al., 1992) 179
6.2.3.3 Company # 2 (Catroux, Rougier et al. 1993) 179
6.2.4 Compilation of data on predictive capacity of the test method 180
6.2.4.1 Description & rational for the Prediction Model(s) applied and statistical approaches used 180
6.2.4.2 Description of performance compared to reference and eventually, to the human situation for each study 180
6.2.4.3 Discussions 181
6.3 Attempt to combine the data using weigh-of-evidence approaches 181

7. Applicability Domain (Module 6) 183

8. Supporting materials 184

8.1 Relevant publications, other scientific reports and reviews 184

List of Tables

Table 1.1 Search items returned in Toxnet and Pubmed 2

Table 1.2 Brief description of data collected with some examples..... 8

Table 2.1.3 Summary of events involved in chemical-induced eye irritation *in vivo*. Text in italics represents irreversible responses. 14

Table 2.2.a Major components of the protocols 17

Table 2.2.b Major components of the protocols 18

Table 2.2.c Major components of the protocols 19

Table 2.2.1.1.a Silicon Microphysiometer data for 11 surfactant-containing materials from Company # 1 24

Table 2.2.1.1.b Cytosensor Microphysiometer data for 11 surfactant-containing materials from Company # 1 24

Table 2.2.3.a Silicon Microphysiometer studies with similar protocols..... 27

Table 2.2.3.b Cytosensor Microphysiometer studies with similar protocols..... 27

Table 2.2.4 Physicochemical properties of test materials and their compatibility with the CM assay for eye irritation 28

Table 3.1 Table presenting the results and relevant information pertinent to within-laboratory reproducibility for studies where raw data are available. 32

Table 3.2.2.1 Within-laboratory reproducibility of SM from Bruner, Miller et al. 1991 using human keratinocytes and a 320 second exposure..... 34

Table 3.2.2.2 Within-laboratory reproducibility from the Company # 2 study of Catroux, Rougier et al. 1993 which used the SM with a 400 sec exposure time 35

Table 3.2.2.3.a Within-laboratory reproducibility of SM from archived data originally obtained at Company # 4 and created for the CTFA Phase III study (Gettings, Lordo et al. 1996). The protocol utilized the SM and a 500 second exposure. 36

Table 3.2.2.3.b Within-laboratory reproducibility of the positive control at Company # 4 during the CTFA Phase III study (Gettings, Lordo et al. 1996)..... 37

Table 3.2.2.4 Within-laboratory reproducibility of CM from archived data that was originally obtained at Company # 4 for the EC/HO study (Balls, Botham et al. 1995). The protocol utilized the CM using transwells and an 810 second exposure time. At least triplicate runs were performed. Surfactants are highlighted. N=35. 39

Table 3.2.2.5.a Surfactant Materials – COLIPA Intralaboratory variability of CM from archived Company # 4 data created for the COLIPA study for surfactant materials (Brantom, Bruner et al. 1997; Harbell, Osborne et al. 1999). The protocol utilized L929 cells and an 810 second exposure. N=8 for surfactant formulations. N=12 for surfactant chemicals. 41

Table 3.2.2.5.b Non-Surfactant Materials – COLIPA Intralaboratory variability of CM from archived Company # 4 data created for the COLIPA study for non-surfactant materials (Brantom, Bruner et al. 1997; Harbell, Osborne et al. 1999). The protocol utilized L929 cells and an 810 second exposure. N=2 for non-surfactant formulations. N=8 for non-surfactant chemicals. 42

Table 3.2.2.5.c Surfactant Materials – COLIPA Within-laboratory reproducibility of CM from archived Company # 5 data created for the COLIPA study for surfactant materials (Brantom, Bruner et al. 1997; Harbell, Osborne et al. 1999). The protocol utilized L929 cells and an 810 second exposure. N=7 for surfactant formulations. N=10 for surfactant chemicals. 43

Table 3.2.2.5.d Non-Surfactant Materials – COLIPA Within-laboratory reproducibility of CM from archived CellTox AB data created for the COLIPA study for surfactant materials (Brantom, Bruner et al. 1997; Harbell, Osborne et al. 1999). The protocol utilized L929 cells and an 810 second exposure. N=2 for non-surfactant formulations. N=7 for non-surfactant chemicals. 44

Table 3.2.2.6.a SM data from a study to determine a conversion factor for changing SM (500 sec exposure) values to CM (810 sec exposure) values. N = 11 materials. 46

Table 3.2.2.6.b CM data from a study to determine a conversion factor for changing SM (500 sec exposure) values to CM (810 sec exposure) values. N = 11 materials. 47

Table 3.2.2.7.a Surfactant materials - Comparison of the MRD_{50} values for testing conducted approximately 21 months apart. N = 11 surfactant materials..... 48

Table 3.2.2.7.b Non-surfactant materials - Comparison of the MRD_{50} values for testing conducted approximately 21 months apart. N = 9 non-surfactant chemicals. 48

Table 3.2.2.8 Positive Control Data of SLS completed at Company # 3..... 50

Table 3.2.3 Operator variability assessed using the positive control data at Company # 3. N = 1 material. 51

Table 4.1.a Results from Company # 4 and Company # 5 laboratories for the COLIPA study demonstrating the transferability of the Cytosensor Microphysiometer protocol. N = 10 surfactant chemicals, N = 7 surfactant formulations, N = 7 Non-surfactant chemicals, and N = 2 non-surfactant formulations. 56

Table 4.1.b Major revisions to the Company # 4 SOP 57

Table 4.2.a Equipment for cell culture 58

Table 4.2.b Specialized assay equipment 58

Table 4.3 Example of the MRD_{50} values for SLS obtained during the training of technicians at Company # 3 60

Table 5.1 Table presenting the relevant results and information for studies where raw data are available 62

Table 5.2.1.a Surfactant based formulations and mixtures - Interlaboratory reproducibility of SM results from Bagley, Bruner et al. 1992 study. The number of replicates for each lab is unknown. N = 21 surfactant formulations. 64

Table 5.2.1.b Surfactant ingredients - Interlaboratory reproducibility of SM results from Bagley, Bruner et al. 1992 study. The number of replicates for each lab is unknown. N = 9 surfactant chemicals. 65

Table 5.2.1.c Non-surfactant ingredients – Interlaboratory reproducibility of SM results from Bagley, Bruner et al. 1992 study. The number of replicates for each lab is unknown. N = 2 non-surfactant chemicals. 65

Table 5.2.2.1 Decisions of the laboratories involved in the EC/HO study as to whether individual test materials were compatible with testing in the CM. 66

Table 5.2.2.2.a Surfactant Materials - Between-laboratories reproducibility of CM results from EC/HO study. The number of replicates for each lab is unknown. N = 12 surfactant chemicals. 68

Table 5.2.2.2.b Non-surfactant materials - Between-laboratories reproducibility of CM results from EC/HO study. The number of replicates for each lab is unknown. N = 48 non-surfactant chemicals. 68

Table 5.2.2.2.c Correlation matrix of alternative CM test scores from individual laboratories for the EC/HO study. From BIBRA project report 1367..... 71

Table 5.2.2.3.a Surfactant Materials - EU, GHS, and EPA classifications based on Cytosensor MRD_{50} values from EC/HO study. Cut-off values from Figures 6.1.3.1.a, 6.1.3.1.b, and 6.1.3.1.c were used. The number of replicates for each lab is unknown. N = 12 surfactant materials. 73

Table 5.2.2.3.b Non-surfactant Materials – EU, GHS, and EPA classifications based on Cytosensor MRD_{50} values from EC/HO study. Cut-off values from Figures 6.1.3.1.a, 6.1.3.1.b, and 6.1.3.1.c were used. The number of replicates for each lab is unknown. N = 48 non-surfactant materials. 74

Table 5.2.2.3.c Surfactant Materials – Agreement table for EU, GHS, and EPA classifications based on Cytosensor MRD_{50} values for the EC/HO study. 76

Table 5.2.2.3.d Non-Surfactant Materials – Agreement table for EU, GHS, and EPA classifications based on Cytosensor MRD_{50} values for the EC/HO study. 76

Table 5.2.3.1 Decisions of the laboratories involved in the COLIPA study as to whether individual test materials were compatible with testing in the CM. 77

Table 5.2.3.2.a Surfactant Materials - Between-laboratories reproducibility of Cytosensor Microphysiometer results from COLIPA study. N = 13 surfactant chemicals. 78

Table 5.2.3.2.b Surfactant based formulations and mixtures - Between-laboratories reproducibility of Cytosensor Microphysiometer results from COLIPA study. N = 22 surfactant formulations..... 79

Table 5.2.3.2.c Non-Surfactants, ingredients, and mixtures - Between-laboratories reproducibility of Cytosensor Microphysiometer results from COLIPA study. N = 10 non-surfactant chemicals and N = 10 non-surfactant formulations... 80

Table 5.2.3.3.a Surfactant Materials - Between-laboratories reproducibility of Cytosensor Microphysiometer hazard classifications from COLIPA study. Cut-off values are based on Figures 6.1.3.3.d, 6.1.3.3.e, and 6.1.3.3.f. N = 14 surfactant materials. 82

Table 5.2.3.3.b Surfactant based formulations and mixtures - Between-laboratories reproducibility of Cytosensor Microphysiometer hazard classifications from COLIPA study. Cut-off values are based on Figures 6.1.3.3.d, 6.1.3.3.e, and 6.1.3.3.f. N = 22 surfactant based formulations. 82

Table 5.2.3.3.c Non-Surfactants, ingredients, and mixtures - Between-laboratories reproducibility of Cytosensor Microphysiometer hazard

classifications from COLIPA study. Cut-off values are based on Figures 6.1.3.3.a, 6.1.3.3.b, and 6.1.3.3.c. N = 10 surfactant chemicals and 10 surfactant materials..... 83

Table 5.2.3.3.d Surfactant Materials - Between-laboratories reproducibility of Cytosensor Microphysiometer hazard classifications agreement from COLIPA study..... 83

Table 5.2.3.3.e Surfactant based formulations and mixtures - Between-laboratories reproducibility of Cytosensor Microphysiometer hazard classifications agreement from COLIPA study..... 83

Table 5.2.3.3.f Non-Surfactants, ingredients, and mixtures - Between-laboratories reproducibility of Cytosensor Microphysiometer hazard classifications agreement from COLIPA study..... 84

Table 5.2.4.a Between laboratories reproducibility correlation coefficients for 14 common test materials tested in the EC/HO (30-33) and COLIPA (27-28) studies. Lab 27 and 31 are the same laboratory (Harbell, Osborne et al. 1999). 84

Table 5.2.4.b Surfactant materials - Between laboratories reproducibility for 11 test materials in common for the EC/HO (Labs 30-33) and COLIPA (Company # 4 and Company # 5) studies. Company # 4 (Lab 27) and SM31 are the same laboratory. N = 11 surfactant materials. 86

Table 5.2.4.c Non-surfactant materials - Between laboratories reproducibility for 9 test materials in common for the EC/HO (Labs 30-33) and COLIPA (Company # 4 and Company # 5) studies. Company # 4 (Lab 27) and SM31 are the same laboratory. N = 9 non-surfactant materials..... 86

Table 5.2.4.d Surfactant Materials - Between-laboratories reproducibility of Cytosensor Microphysiometer hazard classifications for test materials in common for the EC/HO (Labs 30-33) and COLIPA (Company # 4 and Company # 5) studies. Company # 4 (Lab 27) and SM31 are the same laboratory. Cut-off values from Figures 6.1.3.4.1.b, 6.1.3.4.1.c, 6.1.3.4.2.b, 6.1.3.4.2.c, 6.1.3.4.3.b, and 6.1.3.4.3.c were applied. N = 11 surfactant materials..... 87

Table 5.2.4.e Non-Surfactant Materials - Between-laboratories reproducibility of Cytosensor Microphysiometer hazard classifications for test materials in common for the EC/HO (Labs 30-33) and COLIPA (Company # 4 and Company # 5) studies. Company # 4 (Lab 27) and SM31 are the same laboratory. Cut-off values from Figures 6.1.3.4.a, 6.1.3.4.b, and 6.1.3.4.c were applied. N = 9 non-surfactant materials..... 88

Table 5.2.4.f Surfactant Materials - Between-laboratories reproducibility of Cytosensor Microphysiometer hazard classifications agreement for test materials

in common for the EC/HO (Labs 30-33) and COLIPA (Company # 4 and Company # 5) studies. Company # 4 (Lab 27) and SM31 are the same laboratory. 90

Table 5.2.4.g Non-Surfactant Materials - Between-laboratories reproducibility of Cytosensor Microphysiometer hazard classifications agreement for test materials in common for the EC/HO (Labs 30-33) and COLIPA (Company # 4 and Company # 5). Company # 4 (Lab 27) and SM31 are the same laboratory. 90

Table 5.2.5 Between-laboratories reproducibility of Cytosensor Microphysiometer hazard classifications for test materials in common for the CTFA and COLIPA studies. Hazard classifications are based on *in vivo* Draize data..... 92

Table 6.0 Subdivision of each 6 rabbit test group into 20 three rabbit test groups followed by separate hazard classification for each subgroup. Numbers in bold, shaded areas represent results from test materials where the subgroups differed in their hazard classification. Data from the CTFA Phase III study. N = 25 materials..... 96

Table 6.1.1 Table presenting the relevant information for each study where raw data are available..... 100

Table 6.1.2 Table presenting the *in vivo* reference data available..... 102

Table 6.1.3.1.a Summary of the EC/HO study (Balls, Botham et al. 1995). Surfactant materials are highlighted. N = 20 non-surfactant materials and N = 11 surfactant materials..... 104

Table 6.1.3.1.b Identification of the six R41 materials which were underpredicted by EU classification. The lettering system remains the same for the GHS and EPA scatterplots..... 105

Table 6.1.3.1.c EC/HO - Contingency table depicting the concordance and predictivity of the CM assay for EU hazard classifications when the cut-off values shown in Figure 6.1.3.1.a are applied. N = 31 materials..... 107

Table 6.1.3.1.d EC/HO - Contingency table depicting the concordance and predictivity of the CM assay for GHS hazard classifications when the cut-off values shown in Figure 6.1.3.1.b are applied..... 108

Table 6.1.3.1.e EC/HO - Contingency table depicting the concordance and predictivity of the CM assay for EPA hazard classifications when the cut-off values shown in Figure 6.1.3.1.c are applied. N = 27 materials..... 108

Table 6.1.3.1.f EC/HO - Concordance table for severe irritants versus the rest and non-irritants versus the rest. Cut-off values from Figures 6.1.3.1.a, 6.1.3.1.b,

and 6.1.3.1.c were applied. N = 31 materials (EU & GHS) and N = 27 materials (EPA)..... 109

Table 6.1.3.1.g EC/HO Surfactants - Contingency table depicting the concordance and predictivity of the CM assay for EU hazard classifications when the cut-off values shown in Figure 6.1.3.1.d are applied. N = 11 materials. 112

Table 6.1.3.1.h EC/HO Surfactants - Contingency table depicting the concordance and predictivity of the CM assay for GHS hazard classifications when the cut-off values shown in Figure 6.1.3.1.e are applied. N = 11 materials. 113

Table 6.1.3.1.i EC/HO - SurfactantsContingency table depicting the concordance and predictivity of the CM assay for EPA hazard classifications when the cut-off values shown in Figure 6.1.3.1.f are applied. N = 10 materials. 113

Table 6.1.3.1.j EC/HO Surfactants - Concordance table for severe irritants versus the rest and non-irritants versus the rest. Cut-off values from Figures 6.1.3.1.d, 6.1.3.1.e, and 6.1.3.1.f were applied. N = 11 materials (EU & GHS) and N = 10 materials (EPA) 114

Table 6.1.3.2.a Summary of CTFA Phase III study (Gettings, Lordo et al. 1996). N = 25 materials. 116

Table 6.1.3.2.b CTFA - Contingency table depicting the concordance and predictivity of the CM assay for EU hazard classifications when the cut-off values shown in Figure 6.1.3.2.a are applied. N = 25 materials. 119

Table 6.1.3.2.c CTFA - Contingency table depicting the concordance and predictivity of the CM assay for GHS hazard classifications when the cut-off values shown in Figure 6.1.3.2.b are applied. N = 25 materials. 119

Table 6.1.3.2.d CTFA - Contingency table depicting the concordance and predictivity of the CM assay for EPA hazard classifications when the cut-off values shown in Figure 6.1.3.2.c are applied. N = 25 materials..... 120

Table 6.1.3.2.e CTFA - Concordance table for severe irritants versus the rest and non-irritants versus the rest. Cut-off values from Figures 6.1.3.2.a, 6.1.3.2.b, and 6.1.3.2.c were applied. N = 25 materials. 121

Table 6.1.3.3.a Summary of the COLIPA study which includes average values from Company # 4 and Company # 5 laboratories (Brantom, Bruner et al. 1997). Surfactant materials are highlighted. N = 10 non-surfactant materials and N = 19 surfactant materials. 122

Table 6.1.3.3.b COLIPA - Contingency table depicting the concordance and predictivity of the CM assay for EU hazard classifications when the cut-off values shown in Figure 6.1.3.3.a are applied. N = 26 materials..... 126
Table 6.1.3.3.c COLIPA - Contingency table depicting the concordance and predictivity of the CM assay for GHS hazard classifications when the cut-off values shown in Figure 6.1.3.3.b are applied. N = 26 materials..... 126

Table 6.1.3.3.d COLIPA - Contingency table depicting the concordance and predictivity of the CM assay for EPA hazard classifications when the cut-off values shown in Figure 6.1.3.3.c are applied. N = 25 materials..... 127

Table 6.1.3.3.e COLIPA - Concordance table for severe irritants versus the rest and non-irritants versus the rest. Cut-off values from Figures 6.1.3.3.a, 6.1.3.3.b, and 6.1.3.3.c were applied. N = 26 materials (EU & GHS) and N = 25 materials (EPA)..... 128

Table 6.1.3.3.f COLIPA Surfactant and surfactant containing materials - Contingency table for depicting the concordance and predictivity of the CM assay for EU hazard classifications when the cut-off values shown in Figure 6.1.3.3.d are applied. N = 17 surfactant materials. 131

Table 6.1.3.3.g COLIPA Surfactant and surfactant containing materials - Contingency table depicting the concordance and predictivity of the CM assay for GHS hazard classifications when the cut-off values shown in Figure 6.1.3.3.e are applied. N = 17 surfactant materials..... 132

Table 6.1.3.3.h COLIPA Surfactant and surfactant containing materials - Contingency table depicting the concordance and predictivity of the CM assay for EPA hazard classifications when the cut-off values shown in Figure 6.1.3.3.f are applied. N = 17 surfactant materials..... 132

Table 6.1.3.3.i COLIPA Surfactant and surfactant containing materials - Concordance table for surfactant and surfactant containing matierlas for severe irritants versus the rest and non-irritants versus the rest. Cut-off values from Figures 6.1.3.3.d, 6.1.3.3.e, and 6.1.3.3.f were applied. N = 17 surfactant materials..... 133

Table 6.1.3.4.a Surfactant Chemicals from the EC/HO, COLIPA, and CTFA studies. There are 21 total materials; 13 unique chemical:concentration combinations, and 6 totally unique surfactants. N = 21 surfactant chemicals..... 135

Table 6.1.3.4.b Surfactant Formulations from the EC/HO, COLIPA, and CTFA studies. There are 32 test materials; most likely all are unique. N = 32 surfactant formulations..... 136

Table 6.1.3.4.c Non-surfactant Chemicals from the EC/HO, COLIPA, and CTFA studies. There are 27 total materials; 21 unique chemical:concentration combinations, and 19 totally unique chemicals. N = 27 non-surfactant chemicals. 137

Table 6.1.3.4.d Non-surfactant Formulations from the EC/HO, COLIPA, and CTFA studies. N = 2 non-surfactant formulations. 138

Table 6.1.3.4.e Comparison of animal data for the CTFA and COLIPA studies. Liquid Soap 1 and Shampoo # 1 used in the COLIPA study were formulated at 25% the strength used in the CTFA study, but were tested without dilution as was done in the CTFA study. Thus the actual tested concentrations of the formulations were the same. 138

Table 6.1.3.4.1.a Combined studies – Non-surfactant chemicals and formulations - Contingency table depicting the concordance and predictivity of the CM assay for EU hazard classifications when the cut-off values from Figure 6.1.3.4.1.a are applied. N = 29 non-surfactant materials. 143

Table 6.1.3.4.1.b Combined studies – Non-surfactant chemicals - Contingency table depicting the concordance and predictivity of the CM assay for EU hazard classifications when the cut-off values from Figure 6.1.3.4.1.b are applied. N = 27 non-surfactant chemicals. 143

Table 6.1.3.4.1.c Combined studies – Surfactant chemicals - Contingency table depicting the concordance and predictivity of the CM assay for EU hazard classifications when the cut-off values from Figure 6.1.3.4.1.c are applied. N = 21 surfactant chemicals. 143

Table 6.1.3.4.1.d Combined studies – Surfactant containing formulations - Contingency table depicting the concordance and predictivity of the CM assay for EU hazard classifications when the cut-off values from Figure 6.1.3.4.1.d are applied. N = 32 surfactant formulations. 144

Table 6.1.3.4.1.e Combined studies – Surfactant Chemicals and Formulations - Contingency table depicting the concordance and predictivity of the CM assay for EU hazard classifications when the cut-off values from Figure 6.1.3.4.1.e are applied. N = 53 surfactant materials. 144

Table 6.1.3.4.2.a Combined studies – Non-surfactant chemicals and formulations - Contingency table depicting the concordance and predictivity of the CM assay for GHS hazard classifications when the cut-off values from Figure 6.1.3.4.2.a are applied. N = 29 non-surfactant materials. 148

Table 6.1.3.4.2.b Combined studies – Non-surfactant chemicals - Contingency table depicting the concordance and predictivity of the CM assay for GHS hazard

classifications when the cut-off values from Figure 6.1.3.4.2.b are applied. N = 27 non-surfactant chemicals..... 149

Table 6.1.3.4.2.c Combined studies – Surfactant chemicals - Contingency table depicting the concordance and predictivity of the CM assay for GHS hazard classifications when the cut-off values from Figure 6.1.3.4.2.c are applied. N = 21 surfactant chemicals..... 149

Table 6.1.3.4.2.d Combined studies – Surfactant containing formulations - Contingency table depicting the concordance and predictivity of the CM assay for GHS hazard classifications when the cut-off values from Figure 6.1.3.4.2.d are applied. N = 32 surfactant formulations..... 149

Table 6.1.3.4.2.e Combined studies – Surfactants and surfactant containing materials - Contingency table depicting the concordance and predictivity of the CM assay for GHS hazard classifications when the cut-off values from Figure 6.1.3.4.2.e are applied. N = 53 surfactant materials. 150

Table 6.1.3.4.3.a Combined studies – Non-surfactant chemicals and formulations - Contingency table depicting the concordance and predictivity of the CM assay for EPA hazard classifications when the cut-off values from Figure 6.1.3.4.3.a are applied. N = 25 non-surfactant materials. 154

Table 6.1.3.4.3.b Combined studies – Non-surfactant chemicals - Contingency table depicting the concordance and predictivity of the CM assay for EPA hazard classifications when the cut-off values from Figure 6.1.3.4.3.b are applied. N = 23 non-surfactant chemicals..... 155

Table 6.1.3.4.3.c Combined studies – Surfactant chemicals - Contingency table depicting the concordance and predictivity of the CM assay for EPA hazard classifications when the cut-off values from Figure 6.1.3.4.3.c are applied. N = 20 surfactant chemicals..... 155

Table 6.1.3.4.3.d Combined studies – Surfactant containing formulations - Contingency table depicting the concordance and predictivity of the CM assay for EPA hazard classifications when the cut-off values from Figure 6.1.3.4.3.d are applied. N = 32 surfactant formulations..... 155

Table 6.1.3.4.3.e Combined studies - Surfactant Chemicals and Formulations - Contingency table depicting the concordance and predictivity of the CM assay for EPA hazard classifications when the cut-off values from Figure 6.1.3.4.3.e are applied. N = 52 surfactant materials..... 156

Table 6.1.3.4.f Combined studies - Concordance for severe irritants versus the rest and non-irritants versus the rest for the combined studies. Cut-off values from Figures 6.1.3.4.1.a – 6.1.3.4.1.e were applied. N = 29 non-surfactant

materials, 27 non-surfactant chemicals, 21 surfactant chemicals, 32 surfactant formulations, and 53 surfactant materials. 157

Table 6.1.3.4.g Combined studies - Concordance for severe irritants versus the rest and non-irritants versus the rest for the combined studies. Cut-off values from Figures 6.1.3.4.2.a – 6.1.3.4.2.e were applied. N = 29 non-surfactant materials, 27 non-surfactant chemicals, 21 surfactant chemicals, 32 surfactant formulations, and 53 surfactant materials. 158

Table 6.1.3.4.h Combined studies - Concordance for severe irritants versus the rest and non-irritants versus the rest for the combined studies. Cut-off values from Figures 6.1.3.4.3.a – 6.1.3.4.3.e were applied. N = 25 non-surfactant materials, 23 non-surfactant chemicals, 20 surfactant chemicals, 32 surfactant formulations, and 52 surfactant materials. 159

Table 6.1.3.5.a Summary of Company # 1 study. N = 76 materials. 161

Table 6.1.3.5.b Company # 1 - Contingency table depicting the concordance and predictivity of the CM assay for EU hazard classifications when the cut-off values from Figure 6.1.3.5.a are applied. N = 76 materials. 167

Table 6.1.3.5.c Company # 1 - Contingency table depicting the concordance and predictivity of the CM assay for GHS hazard classifications when the cut-off values from Figure 6.1.3.5.b are applied. N = 76 materials. 167

Table 6.1.3.5.d Company # 1 - Contingency table depicting the concordance and predictivity of the CM assay for EPA hazard classifications when the cut-off values from Figure 6.1.3.5.c are applied. N = 76 materials..... 168

Table 6.1.3.5.e Company # 1- Concordance table for surfactant and surfactant containing materials for severe irritants versus the rest and non-irritants versus the rest for the Company # 1 study. Cut-off values from Figures 6.1.3.5.a, 6.1.3.5.b, and 6.1.3.5.c were applied. N = 76 materials..... 169

Table 6.2.1 Table presenting the relevant information for each study where raw data are not available 177

Table 6.2.2 Table presenting the *in vivo* reference data available 178

List of Figures

Figure 2.1.3.a Factors that impact exposure to the eye 11

Figure 2.1.3.b Summary of the Depth of Injury Model 12

Figure 2.1.3.c Example of the metabolic rate data as a function of surfactant type and concentration 13

Figure 2.2.1.a Diagram of the operating components of the silicon microphysiometer (Bruner, Miller et al. 1991) 21

Figure 2.2.1.b The original silicon microphysiometer sensor chamber with the coverslip in place (Bruner, Miller et al. 1991) 21

Figure 2.2.1.c Diagram of the operating components of the Cytosensor (Cytosensor Manual) 21

Figure 2.2.1.d The Cytosensor chamber with the transwell in place (Cytosensor Manual) 22

Figure 2.2.1.1 A comparison of data obtained from 11 surfactant-containing products with SM and CM. 25

Figure 3.2.2.3 Regression plot of the relationship between *in vitro* endpoint and MAS for the SM; dashed curves represent 95% prediction bounds (Gettings, Lordo et al. 1996). Error bars represent one standard error. 37

Figure 3.2.2.5.a Graph of the variability of CM data for the two laboratories participating in the COLIPA study. The CV for each test material measurement is plotted against the mean MRD_{50} value for that material. Twenty-nine materials are plotted for Company # 4 and 26 materials for Company # 5 44

Figure 3.2.2.5.b Graph of the coefficient of variation of CM data for the two laboratories participating in the COLIPA study. The CV obtained for each test material at Company # 5 is plotted against the CV obtained for each test material at Company # 4.
45

Figure 3.2.2.7.a Surfactant materials for comparison between inter-laboratory data of COLIPA and EC/HO studies. 49

Figure 3.2.2.7.b Non-surfactant materials for comparison between inter-laboratory data of COLIPA and EC/HO studies. 49

Figure 3.2.2.8 Graph of 10% SLS (positive control) MRD_{50} values obtained at Company # 3 over a 28-month period.....50
Figure 3.3.3.a Comparison of CV and MRD_{50} for the COLIPA, EC/HO, CTFA and the Bagley studies.....53

Figure 5.2.2.2.a Plot of CM test scores (log MRD_{50}) versus individual chemicals for the EC/HO study. From BIBRA project report 1367.....70

Figure 5.2.2.2.b Plot of residuals of CM test scores versus individual chemicals for the EC/HO study. From BIBRA project report 1367.....70

Figure 6.1.3.1.a Results of the EC/HO study related to EU classification. Data points indicate the mean MRD_{50} for the laboratories (2, 3, or 4) that provided data for that chemical. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude. All materials including surfactants are included. 105

Figure 6.1.3.1.b Results of the EC/HO study related to GHS classification. Data points indicate the mean MRD_{50} for the laboratories (2, 3, or 4) that provided data for that chemical. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude. All materials including surfactants are included.....106

Figure 6.1.3.1.c Results of the EC/HO study related to EPA classification Data points indicate the mean MRD_{50} for the laboratories (2, 3, or 4) that provided data for that chemical. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude. All materials including surfactants are included. 106

Figure 6.1.3.1.d Results of only the surfactants from the EC/HO study related to EU classification. Data points indicate the mean MRD_{50} for the laboratories (2, 3, or 4) that provided data for that chemical. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude. 110

Figure 6.1.3.1.e Results of only the surfactants from the EC/HO study related to GHS classification. Data points indicate the mean MRD_{50} for the laboratories (2, 3, or 4) that provided data for that chemical. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude. 111

Figure 6.1.3.1.f Results of only the surfactants from the EC/HO study related to EPA classification. Data points indicate the mean MRD_{50} for the laboratories (2, 3, or 4) that provided data for that chemical. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude. 111

Figure 6.1.3.2.a Results of the CTFA Phase III study related to EU classification. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude. MRD_{50} is reported in converted CM values. 117

Figure 6.1.3.2.b Results of the CTFA Phase III study related to GHS classification. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude. MRD_{50} is reported in converted CM values. 117

Figure 6.1.3.2.c Results of the CTFA Phase III study related to EPA classification. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude. MRD_{50} is reported in converted CM values. 118

Figure 6.1.3.3.a Results of the COLIPA study related to EU classification. Data points indicate the mean MRD_{50} for both laboratories. Only data from test materials which were tested in both laboratories are shown here. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude. 124

Figure 6.1.3.3.b Results of the COLIPA study related to GHS classification. Data points indicate the mean MRD_{50} for both laboratories. Only data from test materials which were tested in both laboratories are shown here. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude. 124

Figure 6.1.3.3.c Results of the COLIPA study related to EPA classification. Data points indicate the mean MRD_{50} for both laboratories. Only data from test materials which were tested in both laboratories are shown here. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude. 125

Figure 6.1.3.3.d Surfactant results of the COLIPA study related to EU classification. Data points indicate the mean MRD_{50} for both laboratories. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude. 129

Figure 6.1.3.3.e Surfactant results of the COLIPA study related to GHS classification. Data points indicate the mean MRD_{50} for both laboratories. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude. 130

Figure 6.1.3.3.f Surfactant results of the COLIPA study related to EPA classification. Data points indicate the mean MRD_{50} for both laboratories. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude. 130

Figure 6.1.3.4.1.a Combined data for all non-surfactant containing chemicals and formulations from the CTFA, EC/HO and COLIPA studies graphed against EU hazard classifications. Proposed MRD_{50} cut-off values of >80 mg/ml to define No Label materials and <3 mg/ml to define Category R41 materials are shown. There are 29 total materials; 23 unique chemical:concentration combinations, and 21 totally unique chemicals. 139

Figure 6.1.3.4.1.b Combined data for non-surfactant chemicals from the CTFA, EC/HO and COLIPA studies graphed against EU hazard classifications. Proposed MRD_{50} cut-off values of >80 mg/ml to define No Label materials and <3 mg/ml to define Category R41 materials are shown. There are 27 total materials; 21 unique chemical:concentration combinations, and 19 totally unique chemicals. 140

Figure 6.1.3.4.1.c Combined data for surfactant chemicals from the CTFA, EC/HO and COLIPA studies graphed against EU hazard classifications. Proposed MRD_{50} cut-off values of >10 mg/ml to define No Label materials and <2 mg/ml to define Category R41 materials are shown. There are 21 total materials; 13 unique chemical:concentration combinations, and 6 totally unique chemicals. 140

Figure 6.1.3.4.1.d Combined data for surfactant formulations from the CTFA, EC/HO and COLIPA studies graphed against EU hazard classifications. Proposed MRD_{50} cut-off values of >10 mg/ml to define No Label materials and <2 mg/ml to define Category R41 materials are shown. There are 32 totally unique chemicals. The assumption is that the six reformulated test materials for the COLIPA study are different from similar test materials used in the CTFA study. 141

Figure 6.1.3.4.1.e Combined data for surfactants and surfactant containing materials from the CTFA, EC/HO and COLIPA studies graphed against EU hazard classifications. Proposed MRD_{50} cut-off values of >10 mg/ml to define No Label materials and <2 mg/ml to define R41 materials are shown. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude. There are 53 total materials; 45 unique chemical:concentration combinations, and 38 totally unique chemicals/formulations. 141

Figure 6.1.3.4.2.a Combined data for all non-surfactant containing chemicals and formulations from the CTFA, EC/HO and COLIPA studies graphed against GHS hazard classifications. Proposed MRD_{50} cut-off values of >80 mg/ml to define No Label materials and <3 mg/ml to define Category 1 materials are shown. There are 29 total materials; 23 unique chemical:concentration combinations, and 21 totally unique chemicals. 146

Figure 6.1.3.4.2.b Combined data for non-surfactant containing chemicals from the CTFA, EC/HO and COLIPA studies graphed against GHS hazard classifications. Proposed MRD_{50} cut-off values of >80 mg/ml to define No Label materials and <3 mg/ml to define Category 1 materials are shown. There are 27 total materials; 21 unique chemical:concentration combinations, and 19 totally unique chemicals. 146

Figure 6.1.3.4.2.c Combined data for surfactant containing chemicals from the CTFA, EC/HO and COLIPA studies graphed against GHS hazard classifications. Proposed MRD_{50} cut-off values of >10 mg/ml to define No Label materials and <2 mg/ml to define Category 1 materials are shown. There are 21 total materials; 13 unique chemical:concentration combinations, and 6 totally unique chemicals. 147

Figure 6.1.3.4.2.d Combined data for surfactant containing formulations from the CTFA, EC/HO and COLIPA studies graphed against GHS hazard classifications. Proposed MRD_{50} cut-off values of >10 mg/ml to define No Label materials and <2 mg/ml to define Category 1 materials are shown. There are 32 totally unique chemicals. The assumption is that the six reformulated test materials for the COLIPA study are different from similar test materials used in the CTFA study. 147

Figure 6.1.3.4.2.e Combined data from the CTFA, EC/HO and COLIPA studies graphed against GHS hazard classifications. Proposed MRD_{50} cut-off values of >10 mg/ml to define No Label materials and <2 mg/ml to define category 1 materials are shown. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude. There are 53 total materials; 45 unique chemical:concentration combinations, and 38 totally unique chemicals/formulations. 148

Figure 6.1.3.4.3.a Combined data for all non-surfactant chemicals and formulations from the CTFA, EC/HO and COLIPA studies graphed against EPA hazard classifications. Proposed MRD_{50} cut-off values of >80 mg/ml to define category IV materials and <3 mg/ml to define category I materials are shown. There are 25 total materials; 20 unique chemical:concentration combinations, and 18 totally unique chemicals. 152

Figure 6.1.3.4.3.b Combined data for non-surfactant chemicals from the CTFA, EC/HO and COLIPA studies graphed against EPA hazard classifications. Proposed MRD_{50} cut-off values of >80 mg/ml to define category IV materials and <3 mg/ml to define category I materials are shown. There are 23 total materials; 18 unique chemical:concentration combinations, and 16 totally unique chemicals. 152

Figure 6.1.3.4.3.c Combined data for surfactant chemicals from the CTFA, EC/HO and COLIPA studies graphed against EPA hazard classifications. Proposed MRD_{50} cut-off values of >80 mg/ml to define category IV materials and <2 mg/ml to define category I materials are shown. There are 20 total materials; 12 unique chemical:concentration combinations, and 6 totally unique chemicals. 153

Figure 6.1.3.4.3.d Combined data for surfactant formulations from the CTFA, EC/HO and COLIPA studies graphed against EPA hazard classifications. Proposed MRD_{50} cut-off values of >80 mg/ml to define category IV materials and <2 mg/ml to define category I materials are shown. There are 32 totally unique chemicals. The assumption is that the six reformulated test materials for the COLIPA study are different from similar test materials used in the CTFA study..... 153

Figure 6.1.3.4.3.e Combined data for all surfactants and surfactant containing materials from the CTFA, EC/HO and COLIPA studies graphed against EPA hazard classifications. Proposed MRD_{50} cut-off values of >80 mg/ml to define Category IV materials and <2 mg/ml to define Category I materials are shown. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude. There are 52 total materials; 44 unique chemical:concentration combinations, and 38 totally unique chemicals/formulations..... 154

Figure 6.1.3.5.a Results from 76 materials from Company #1 related to EU classification. For the four materials in Table 6.1.3.5.a which had 2 independent animal tests only the most irritating animal scores were used in this figure..... 164

Figure 6.1.3.5.b Results from 76 materials from Company #1 related to GHS classification. For the four materials in Table 6.1.3.5.b which had 2 independent animal tests only the most irritating animal scores were used in this figure..... 165

Figure 6.1.3.5.c Results from 76 materials from Company #1 related to EPA classification. For the four materials in Table 6.1.3.5.c which had 2 independent animal tests only the most irritating animal scores were used in this figure..... 166

List of Annexes

ANNEX A (Protocols) **A1**
Current Protocol from Company # 3 ..... A3
COLIPA Protocol A13
INVITOX Protocol (IP-97) A25
INVITOX Protocol (IP-102) A29
EC/HO Protocol A35

ANNEX B (Physiochemical Properties of Chemicals) **B1**

ANNEX C (Physiochemical Properties of Formulations) **C1**

ANNEX D (Chemicals and Formulations) **D1**
CTFA Phase III Study Formulations D3
COLIPA Study Formulations D11
EC/HO Study Chemicals by Class D21

ANNEX E (Study and Test Material Relationship) **E1**

ANNEX F (Study Reports) **F1**
CTFA Phase III Study Report F3
COLIPA Study Report and Decoding Information F13
Report for Company # 1 Comparing Silicon Microphysiometer and Cytosensor Microphysiometer Data F35

ANNEX G (References Containing Data) **G1**

ANNEX H Raw Cytosensor Data **H1**
Raw Data from Company # 2 ..... H3
EC/HO Raw Data from Company # 3 ..... H7
EC/HO Average Data from Four Labs H13
Company # 3 Positive Control Data H17
Company # 3 Operator Variability H25
COLIPA Raw Data from Company # 5 ..... H29

ANNEX I (CTFA Phase III Study Animal Data) **I1**
CTFA Phase III Study Draize Hazard Classification Spreadsheets I3
CTFA Phase III Study LVET Hazard Classification Spreadsheets I29
CTFA Phase II Study Draize Animal Data I55
CTFA Phase III Study LVET Animal Data– 3 Day Averages I81

ANNEX J (COLIPA Animal Data) **J1**
COLIPA Animal Data Hazard Classification Spreadsheets J3
COLIPA Draize Animal Data J59

ANNEX K (Company # 1 Animal Data) **K1**
Company # 1 Animal Data Hazard Classification Spreadsheets K3
Company # 1 LVET Animal Data K85

ANNEX L (EC/HO Animal Data) **L1**

List of Abbreviations

ATP	Adenosine triphosphate
BRD	Background review document
CAM	Chroioallantoic Membrane Vascular Assay
CM	Cytosensor microphysiometer
CMC	Critical Micelle Concentration
COLIPA	European Cosmetic, Toiletry, and Perfumery Association
CRO	Contract Research Organization
CTFA	U.S. Cosmetics, Toiletries, and Fragrance Association
CV	Coefficient of variance
DNA	Deoxyribonucleic acid
EC/HO	European Commission/British Home Office
ECVAM	European Centre for the Validation of Alternative Methods
EU	European Union
GHS	Globally Harmonized System
GLP	Good laboratory practices
HCE	Human Cornea Epithelium
ICCVAM	Interagency Co-ordinating Committee on the Validation of Alternative Methods
INVITTOX	Compendium of *In vitro* Toxicology protocols maintained by ECVAM
IRAG	U.S. Interagency Regulatory Alternatives Group
LVET	Low volume eye test
MAS	Maximum Average Score
MMAS	Modified Maximum Average Score
MRD_{50}	Metabolic rate decrement of 50%
MT	Management Team
NPV	Negative predictive value
pH	An acidity/alkalinity index; the reciprocal of the hydrogen ion concentration
PPV	Positive predictive value
QC	Quality Control
SD	Standard Deviation
SEM	Standard Error of the Mean
SLS	Sodium lauryl sulfate
SM	Silicon microphysiometer
SOP	Standard Operating Procedures
USEPA	United States Environmental Protection Agency

Acknowledgements

The generous contributions from the following companies and individuals who provided *in vitro* and/or *in vivo* data considered in this Background Review Document are gratefully acknowledged:

The Procter and Gamble Company, Cincinnati, OH USA
Pauline McNamee and Rosemarie Osborne

L'Oreal Corporation, Paris, France
Odile deSilva

Institute for In Vitro Sciences, Inc., Gaithersburg, MD USA
Greg Mun and Hans Raabe

1. Data collection

1.1 Description of the methods used to collect data, including literature search or other sources, and number of studies collected

Retrospective analysis of a method requires a complete search for available data. The method under review here utilizes an instrument which performs real time measurements of the metabolic rate of a cell population. This instrument has existed in two forms, and thus a search for data must include reference to the names of both instruments. The use of the silicon microphysiometer (SM) (the prototype instrument) began in approximately 1989 and the Cytosensor microphysiometer (the commercial instrument) (hereafter referred to as Cytosensor or CM) became available in 1995. In 1993, the sensor chambers for the silicon microphysiometer were changed from the coverslip configuration to the transwell configuration in preparation for the introduction of the commercial instrument. At that point, the standard protocol was developed for the transwell exposures and that protocol has been used for both the transwell-equipped silicon microphysiometer and the commercial Cytosensor. Most of the data provided in this BRD come from the transwell configuration of the silicon microphysiometer and Cytosensor instruments and its assay protocol. The Cytosensor is the instrument in current use.

Several of the authors of this Background Review Document (BRD) have been involved with the SM and subsequently the CM since 1990. Thus, they are aware of many of the previous studies on the evaluation of eye irritation potential. However, a full literature search (conducted through NERAC, Inc., Tolland, CT) was undertaken using "microphysiometer" and "Cytosensor" search terms. The databases searched by NERAC included, but were not limited to, Biobusiness, Biological Abstracts, Medline, Embase, and Life Sciences Collection. As the Cytosensor was primarily intended as a drug development tool, most of the references provided by the search dealt with specific receptor binding assays and were not applicable to this BRD.

In addition, both Toxnet and Pubmed were searched directly, and the following unique references were found. There were no references found in the NERAC search that were different than those found in Toxnet and Pubmed listed below. References that were similar between search terms are listed only once below. Table 1.1 describes the number of unique returns with each keyword for each database. The numbers in bold indicate articles returned with reasonable relevancy. Relevant articles are those defined as either having information on the toxicity of chemicals or formulations, or those giving important background information on the functioning of the SM (or CM) or how cellular changes in metabolic rate could be interpreted.

Table 1.1 Search items returned in Toxnet and Pubmed

Search Term	Database	
	TOXNET	PUBMED
Microphysiometer	**17** (55)	**20** (138)
Cytosensor	**5** (35)	**15** (97)
Silicon Microphysiometer	**8** (22)	**5** (24)

Toxnet:

Parce, J.W., et al., *The Microphysiometer and Its Application in Irritancy Testing. In vitro* Cell Dev Biol, 1990. **26**(3 Part 2): p. 35A.

Bruner, L.H., et al., *Evaluation of Seven In vitro Alternatives for Ocular Safety Testing.* Fundam Appl Toxicol, 1991. **17**(1): p. 136-149.

Bagley, D.M., et al., *An Evaluation of Five Potential Alternatives In vitro to the Rabbit Eye Irritation Test In vivo.* Toxicology *In vitro*, 1992. **6**(4): p. 275-284.

Calvin, G., *New Approaches to the Assessment of Eye and Skin Irritation.* Toxicology Letters, 1992. **64/65**: p. 157-164.

Parce, J.W., *Cells on Silicon Bioassays with a Microphysiometer.* FED AM SOC EXP BIOL, 1992. **6**(1): p. A5.

Catroux, P., et al., *The Silicon Microphysiometer for Testing Ocular Toxicity In vitro.* Toxicol *In vitro*, 1993. **7**(4): p. 465-469.

Harvell, J.D., et al., *An In vivo Correlation with Three In vitro Assays to Assess Skin Irritation Potential.* Journal of Toxicology - Cutaneous and Ocular Toxicology, 1994. **13**(2): p. 171-183.

Hirst, M.A., C.E. Green, and C. Tyson, .A., *Initial Studies of the Effects of Toxic Agents on Hepatocytes Using the Cytosensor Microphysiometer System. In vitro* Toxicology, 1994. **7**(2): p. 136.

Ajilore, O.A. and R.M. Sapolsky, *Application of Silicon Microphysiometery to Tissue Slices: Detection of Metabolic Correlates of Selective Vulnerability.* Brain Research, 1997. **752**(7-2): p. 99-106.

Botham, P., et al., *Cell Function-Based Assays.* Food and Chemical Toxicology, 1997. **35**(1): p. 67-77.

Botham, P., et al., *IRAG Working Group 3. Cell Function-based Assays. Interagency Regulatory Alternatives Group.* Food and Chemical Toxicology, 1997. **35**(1): p. 67-77.

Cao, C.J., et al., *Toxicity of Sea Nettle Toxin to Human Hepatocytes and the Protective Effects of Phosphorylating and Alkylating Agents.* Toxicon, 1998. **36**(2): p. 269-281.

Gronert, K., S.P. Colgan, and C.N. Serhan, *Characterization of Human Neutrophil and Endothelial Cell Ligand-Operated Extracellular Acidification Rate by Microphysiometry: Impact of Reoxygenation.* J Pharmacol Exp Ther, 1998. **285**(1): p. 252-261.

Jordan, R.E., et al., *Activation of the Cloned Human NK3 Receptor in Chinese Hamster Ovary Cells Characterized by the Cellular Acidification Response Using the Cytosensor Microphysiometer.* British Journal of Pharmacology, 1998. **125**(4): p. 761-766.

Cao, C.J., et al., *Cytotoxicity of Organophosphate Anticholinesterases. In vitro* Cell Dev Biol Anim, 1999. **35**(9): p. 493-500.

Cooke, D. and R. O'Kennedy, *Comparison of the Tetrazolium Salt Assay for Succinate Dehydrogenase with the Cytosensor Microphysiometer in the Assessment of Compound Toxicities.* Analytical Biochemistry, 1999. **274**(2): p. 188-194.

Harbell, J.W., et al., *Assessment of the Cytosensor™ Microphysiometer Assay in the COLIPA In vitro Eye Irritation Validation Study.* Toxicology *In vitro*, 1999. **13**: p. 313-323.

Koebe, H.G., et al., *In vitro Toxicology in Hepatocyte Bioreactors-Extracellular Acidification Rate (EAR) in a Target Cell Line Indicates Hepato-activated Transformation of Substrates.* Toxicology, 2000. **154**(1-3): p. 31-44.

Burvall, K., L. Palmberg, and K. Larsson, *Metabolic Activation of A549 Human airway Epithelial Cells by Organic Dust: A Study Based on Microphysiometery.* Life Sciences, 2002. **71**(3): p. 299-309.

Deglmann, C.J., et al., *A New Bioassay Including a Small Scale Hepatocyte Bioreactor for Hepato-mediated Toxicity Testing in a Target Cell Line.* Int J Artif Organs, 2002. **25**(10): p. 975-984.

Silbergeld, E.K., *Neurotoxicology Studies.* CRISP.

Pubmed:

Owicki, J.C. and J.W. Parce, *Bioassays with a Microphysiometer.* Nature, 1990. **344**(6263): p. 271.

Bruner, L.H., et al., *Evaluation of Seven In vitro Alternatives for Ocular Safety Testing.* Fundam Appl Toxicol, 1991. **17**(1): p. 136-149.

McConnell, H.M., et al., *The Cytosensor Microphysiometer: Biological Applications of Silicon Technology.* Science, 1992. **257**(5078): p. 1906-12.

Botham, P., et al., *IRAG Working Group 3. Cell Function-based Assays. Interagency Regulatory Alternatives Group.* Food and Chemical Toxicology, 1997. **35**(1): p. 67-77.

Cao, C.J., et al., *Toxicity of Sea Nettle Toxin to Human Hepatocytes and the Protective Effects of Phosphorylating and Alkylating Agents.* Toxicon, 1998. **36**(2): p. 269-281.

Cao, C.J., et al., *Cytotoxicity of Organophosphate Anticholinesterases. In vitro* Cell Dev Biol Anim, 1999. **35**(9): p. 493-500.

Cooke, D. and R. O'Kennedy, *Comparison of the Tetrazolium Salt Assay for Succinate Dehydrogenase with the Cytosensor Microphysiometer in the Assessment of Compound Toxicities.* Analytical Biochemistry, 1999. **274**(2): p. 188-194.

Ren, X., D. Wang, and H. Li, *Study on Electrochemical Behavior of HL-60 Cells During the Etioposide-inducing Apoptosis.* Zhonghua Yi Xue Za Zhi, 1999. **20**(2): p. 82-84.

Hafner, F., *Cytosensor Microphysiometer: Technology and Recent Applications.* Biosens Bioelectron, 2000. **15**(3-4): p. 149-158.

Koebe, H.G., et al., *In vitro Toxicology in Hepatocyte Bioreactors-Extracellular Acidification Rate (EAR) in a Target Cell Line Indicates Hepato-activated Transformation of Substrates.* Toxicology, 2000. **154**(1-3): p. 31-44.

Chen, Z.W., K. Yang, and Y. Wang, *Microphysiometer-a real-time, Sensitive Method for Evaluation of the Functional Activity of Cells.* Sheng Li Ke Xue Jin Zhan, 2001. **32**(3): p. 243-245.

Luckie, D.B., et al., *CFTR Activation Raises Extracellular pH of NIH/3T3 Mouse Fibroblasts and C127 Epithelial Cells.* J Membr Biol, 2001. **179**(3): p. 275-284.

Burvall, K., L. Palmberg, and K. Larsson, *Metabolic Activation of A549 Human airway Epithelial Cells by Organic Dust: A Study Based on Microphysiometery.* Life Sciences, 2002. **71**(3): p. 299-309.

Cai, B., et al., *Apoptosis-inducing Activity of Extract from Chinese Herb, Albizzia Lucidior I. Nielsen.* Ai Zheng, 2002. **21**(4): p. 373-378.

Deglmann, C.J., et al., *A New Bioassay Including a Small Scale Hepatocyte Bioreactor for Hepato-mediated Toxicity Testing in a Target Cell Line*. Int J Artif Organs, 2002. **25**(10): p. 975-984.

Landwojtowicz, E., P. Nervi, and A. Seelig, *Real-time Monitoring of P-glycoprotein Activation in Living Cells*. Biochemistry, 2002. **41**(25): p. 8050-8057.

Park, T.H. and M.L. Shuler, *Integration of Cell Culture and Microfabrication Technology*. Biotechnol Prog, 2003. **19**(2): p. 243-253.

Wille, K., L.A. Paige, and A.J. Higgins, *Application of the Cytosensor Microphysiometer to Drug Discovery*. Receptors Channels, 2003. **9**(2): p. 125-131.

Eklund, S.E., et al., *A Microphysiometer for Simultaneous Measurement of Changes in Extracellular Glucose, Lactate, Oxygen, and Acidification Rate*. Anal Chem, 2004. **76**(3): p. 519-527.

Erxleben, H.A., et al., *A Novel Approach for Monitoring Extracellular Acidification Rates: Based on Bead Injection Spectophotometry and the Lab-on-valve System*. Analyst, 2004. **129**(3): p. 205-212.

Gatlik-Landwojtowicz, E., P. Aanismaa, and A. Seelig, *The Rate of P-glycoprotein Activation Depends on the Metabolic State of the Cell*. Biochemistry, 2004. **43**(46): p. 14840-14851.

Gatlik-Landwojtowicz, E., P. Aanismaa, and A. Seelig, *Quantification and Characterization of P-glycoprotein-substrate Interactions*. Biochemistry, 2006. **45**(9): p. 3020-3032.

1.1.1 Studies identified outside of database searches

In addition to the references identified by the standard database searches, the authors of this BRD were aware of other articles dealing with the performance of the SM or CM in ocular irritation studies. Those articles are listed below.

Balls, M., P. A. Botham, et al. *The EC/HO International Validation Study on Alternatives to the Draize Eye Irritation Test*. Toxicology *In vitro*, 1995. **9**(6): p. 871-929.

Brantom, P. G., L. H. Bruner, et al. *A Summary Report of the COLIPA International Validation Study on Alternatives to the Draize Rabbit Eye Irritation Test*. Toxicology *In vitro*, 1997. **11**: p. 141-179.

Bruner, L. H., K. M. Miller, et al. *Testing Ocular Irritancy In vitro with the Silicon Microphysiometer*. Toxicology *In vitro*, 1991. **5**: p. 277-284.

Gettings, S. D., R. A. Lordo, et al. *The CTFA Evaluation of Alternatives Program: An Evaluation of In vitro Alternatives to the Draize Primary Eye Irritation Test. (Phase III) Surfactant-based Formulations.* Food Chem Toxicol, 1996. **34**(1): p. 79-117.

Parce, J. W., J. C. Owicki, et al. *Detection of Cell-affecting Agents with a Silicon Biosensor.* Science, 1989. **246**: 243-247.

1.2 Brief description of data collected on overall study management

The major focus of the data collection was on data that provided parallel animal and *in vitro* data. Two major categories are data generated in 3rd party evaluation/validation studies and in-house data from individual companies (e.g., product safety data). To this end, the Cosmetics, Toiletries, and Fragrance Association (CTFA) Phase III study (Gettings, Lordo et al. 1996), European Commission/British Home Office (EC/HO) (Balls, Botham et al. 1995), and COLIPA Eye Irritation Validation study (Brantom, Bruner et al. 1997; Harbell, Osborne et al. 1999) data have been compiled as examples of the first category. The EC/HO and COLIPA studies provide data for the evaluation of between-laboratory consistency as well. For the second category, the largest data set (~80 paired data sets) comes from the in-house data of Company # 1. This corporate data set from Company # 1 uses the LVET as the reference data. The EC/HO, COLIPA, and CTFA use the Draize test as the *in vivo* reference assay. For comparison purposes, the CTFA study tested the materials in their study with both the Low Volume Eye Test (LVET) (Griffith, Nixon et al. 1980; Freeberg, Nixon et al. 1986) and the Draize test (Freeberg, Nixon et al. 1986).

The positive control for both the SM and CM has generally been sodium lauryl sulfate (SLS). Tracking of these results over time gives valuable information concerning the reproducibility of the assay within a laboratory. These data were generated both at Company # 4 (1994-1997) and at Company # 3 (1997-2006). Therefore, a historical control database for each assay has been provided using the in-house information from the archives of Company # 3.

Several studies provided only summary *in vivo* data (i.e., MMAS) and so the analysis of Globally Harmonized System (GHS), European Union (EU) and US Environmental Protection Agency (USEPA) Categories is not possible. The *in vivo* and *in vitro* data from the two Bruner, et al. studies (Bruner, Kain et al. 1991); (Bruner, Miller et al. 1991) are the same and use the standard silicon microphysiometer protocol. Company # 2 has provided summary *in vivo* data on the ingredients tested (Catroux, Rougier et al. 1993). Three data sets were summarized in the Interagency Regulatory Alternatives Group (IRAG) (Brantom, Bruner et al. 1997) report. These include the CTFA Phase III study (Company # 4) (Gettings,

Lordo et al. 1996), the data from Company # 2 (Catroux, Rougier et al. 1993), and early data from Company # 1.

Two additional studies are included to compare the silicon microphysiometer and the Cytosensor instrumentation. The report of Bagley, Bruner et al. 1992 contains summary MAS values only but does compare the MRD_{50} values for the silicon microphysiometer with coverslip (standard protocol with a 500-second exposure at each concentration) and the silicon microphysiometer with transwell (500 second exposure). An unpublished study provided by Company # 1 has been included to compare the MRD_{50} values obtained with the silicon microphysiometer with the glass coverslip (standard protocol) and with the Cytosensor with the transwell (with its standard protocol). This study was performed to develop a translation factor between the SM and CM MRD_{50} values.

Table 1.2 summarizes the studies included in this Background Review Document. Approximately 200 paired data sets with complete *in vivo* and at least summary *in vitro* data are available for review. Additional data sets include summary *in vivo* and *in vitro* data.

Table 1.2 Brief description of data collected with some examples

Sponsor	Management	Chemical / formulation Selection / Coding	Process of data collection and statistical analysis	Standardized Prediction model	Quality of data (*in vivo* / *in vitro*) (GLP/non-GLP)	Standardized protocol available	Format of data available (*in vivo* / *in vitro*)
Company # 1[1] (Bruner, Miller et al. 1991)	Corporate participants	Selected from the corporate database of personal care, household cleaning products, and ingredients; coding unknown	Internal	No	Unknown	Not available for this BRD	Summary (MAS LVET) and mean MRD_{50} values. GLP status of either data set is unknown
Company # 1[1] (Bruner, Kain et al. 1991)	Corporate participants	These chemicals and data are the same as those in the Bruner, et al. 1991 publication	Internal	No	Unknown	Not available for this BRD	Summary (MAS LVET) and mean MRD_{50} values. GLP status of either data set is unknown
Several companies[2] (Bagley, Bruner et al. 1992)	Corporate participants	Selected from household cleaning products, personal care, and cosmetics categories plus ingredients for these categories	Unknown	No	Unknown	Not available for this BRD	Summary MAS (Draize and LVET) and MRD_{50} values (silicon microphysiometer and Cytosensor, both 500 sec exposure).
Company # 2[3] (Catroux, Rougier et al. 1993)	Corporate participants	Selected from the corporate database (ingredients and formulations) coding unknown	Internal	Not prestudy, but one proposed posthoc	Unknown	Not available for this BRD	Summary MAS and MRD_{50} values in publication. More complete data for 20 of the 53 materials obtained for this BRD.
EC/HO[4] (Balls, Botham et al. 1995)	Independent MT; laboratories selected based on experience	Selected by the management team based on the availability of good animal data (ECETOC) and a range of chemical classes/coded	Independent contractor	None	*In vivo* – Unknown *In vitro* – non GLP	Yes, but level of adherence to protocol not documented;	*In vivo* – all *In vitro* – summaries (means) for 3 labs, raw data available from 1 lab

CTFA[5] (Gettings, Lordo et al. 1996)	Independent	Independently selected (based on a range of irritancy) from the categories surfactants and surfactant-containing products, supplied coded	Independent	None	*In vivo* - GLP *In vitro* – non GLP; concurrent testing *in vivo* / *in vitro*	Yes (only one lab running the assay); not available for this BRD	*In vivo* – all *In vitro* – each trial
COLIPA[6] (Brantom, Bruner et al. 1997; Harbell, Osborne et al. 1999)	Independent MT; laboratories selected based on experience	Independent chemical / formulation selection/ coded	Independent	Yes, based on MMAS	*In vivo* – GLP (some concurrent / some historical) *In vitro* – non GLP	Yes	*In vivo* – all *In vitro* – each trial
Company # 3[6 & 7] (Unpublished – in-house positive control data)	Company # 3	SLS positive control Not coded	Concurrent testing with each trial	Defined limits	Generally GLP	Yes (both SM and CM)	MRD_{50} from each trial and summary of data over time
Company # 1[6 & 7] (Unpublished)	Corporate and contract laboratory	Materials tested as part of ongoing products/ingredient safety program / Coded for the testing laboratory	Reports to Company # 1 from the CRO. Analysis conducted in this BRD	Not Available	Generally GLP (*in vitro*)	Yes (both SM and CM)	Complete animal data (LVET) and summary MRD_{50} (derived from three trials)

[1] Performed using the silicon microphysiometer coverslip protocol with a 320-second exposures and normal human epidermal keratinocytes (see (Bruner, Miller et al. 1991)).
[2] Performed using the silicon microphysiometer coverslip protocol and the silicon microphysiometer transwell protocol with a 500-second exposure cycle and L929 cells.
[3] Performed using the silicon microphysiometer transwell protocol with the 400-second exposure cycle and L929 cells.
[4] Performed using the Cytosensor transwell protocol with an 810-second exposure and L929 cells.
[5] Performed using the silicon microphysiometer coverslip protocol with a 300-second exposure and L929 cells.
[6] Performed using the Cytosensor transwell protocol with an 810-second exposure and L929 cells.
[7] Performed using the silicon microphysiometer coverslip protocol with a 500-second exposure and L929 cells.

2. Test Definition (Module 1)

2.1 Rationale for the proposed test method

2.1.1 Intended uses / purpose

Currently the CM is used by industry early in the product development process to screen liquid ingredients for cosmetic, personal care, and household cleaning products. This is then often followed by evaluations of the final formulations for final in-house safety decisions. Data from the CM may be combined with information from other *in vitro* or in silico assays to provide a "weight of evidence" evaluation of the formulation. Information from this assay is generally not combined with animal data in making the final safety decision for the product.

At the time the CM technology was developed, a number of *in vitro* assays such as the Neutral Red Uptake assay were already proposed as potential replacements for the Draize eye irritation test. However, the great advantage of the CM or SM technology was that real time measurements could be made of the cytotoxic response of the target cells as opposed to the 2-3 days or longer time which was required of the existing cytotoxicity assays. Thus, the assay was mainly created not to reveal a completely new endpoint, but rather to provide data in a much shorter time period.

2.1.2 Regulatory rational and applicability

To the best of our knowledge, the CM assay is not currently included in the regulatory scheme of any country. Data are used primarily to evaluate raw materials and formulations where regulatory registration is not required. It has been reviewed informally by regulatory agencies in the US as part of the Interagency Regulatory Alternatives Group (IRAG) evaluation of alternative ocular irritation assays (Botham, Osborne et al. 1997), and it is expected to be included as one of the assays that will be evaluated by the US Environmental Protection Agency (through the Interagency Co-ordinating Committee on the Validation of Alternative Methods (ICCVAM)) as part of a larger initiative to replace the requirement for animal testing to determine the ocular irritation capacity of anti-microbial cleaning formulations. This evaluation is expected to begin in the 2007/2008 time frame.

2.1.3 Scientific basis for the test

Topical applications of chemicals can kill cells in several ways; among these are lysis of membranes, denaturation of proteins, saponification of lipids, and alkylation or other covalent interactions with macromolecules. The first three modes of action kill or damage very rapidly while the last may act rapidly but the evidence of the action may take some time to be manifested (Maurer, Parker et al. 2002). Certain chemical classes are

associated with these modes of action. Surfactants are primarily associated with membrane lysis although cationic surfactants may also act to precipitate proteins and other macromolecules. Organic solvents can act to delipidize and thus lyse membranes as well as denature (coagulate or precipitate) proteins. Acids tend to coagulate or precipitate proteins. Alkalis saponify lipids and denature proteins in a way that tends to allow them to penetrate into the cornea. Bleaches, peroxides, alkylators (e.g. mustards) bind to macromolecules (especially DNA) leading to cell death.

Damage to the eye is a function of the inherent cytotoxicity potential of the chemical or mixture, the effective concentration impacting the tissues and the residence time at that concentration on or in the tissues. The effective exposure is a combination of concentration and time of exposure (Figure 2.1.3.a). For example, a neat organic solvent may have a high cytotoxic potential but if it rapidly evaporates, the effective residence time will be less. Putting a large volume into a closed sac (e.g., lower conjunctival sac of the rabbit eye) will produce a very different effective exposure than a smaller amount placed (or accidentally splashed) onto the open surface of the cornea. Another solvent may have a longer residence time but have its cytotoxic potential rapidly reduced by dilution with tears. In this case, the irritation potential in a species with a low propensity to tear could show much more irritation than in a species with a high propensity to tear. The effective exposure to solids (powders) in the eye is a particular challenge. Powders placed into the conjunctival sac may have a residence time that ranges from minutes to a full day (and longer in some older studies) (Prinsen 2006). Traditional studies of eye irritation potential do not measure or control the effective exposure within or among studies. Thus, efforts to model exposure in alternative test systems are based on best estimates and approximations.

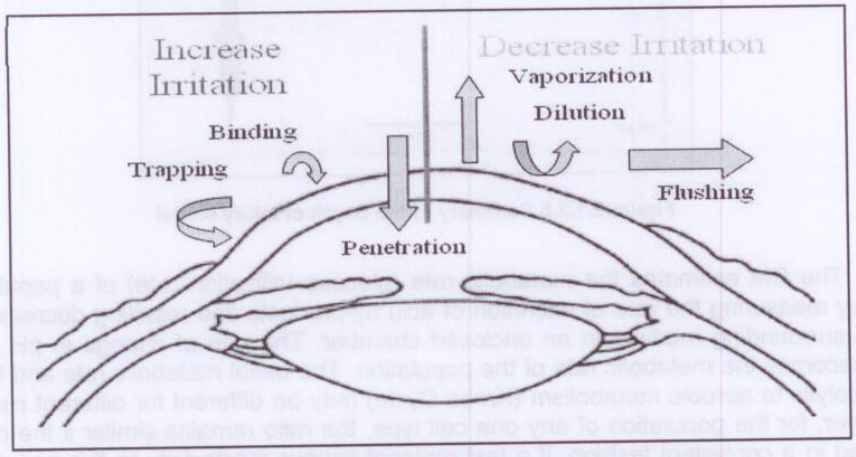


Figure 2.1.3.a Factors that impact exposure to the eye

Mechanistically, this cytotoxicity assay is intended to model the action of the surfactant on the cell membranes of the corneal and conjunctival epithelium where the test article would reside in an *in vivo* exposure. The potency of the surfactant (or surfactant

formulation) *in vivo* is related to the area and number of cell layers that can be lysed during the effective exposure period. More potent (and/or more substantive) surfactants will be more effective at a given concentration and exposure period. Potency can be a function of concentration (e.g., in a formulation) or chemical structure. Thus, a lower concentration of a more potent surfactant or more concentrated formulation would be required to lyse the membranes, and thus kill a given fraction of the cells in the epithelia (both corneal and conjunctival). Expressed another way, a given concentration of a more potent test material should lyse more cells (i.e., greater depth of penetration and injury). Initial depth of injury has been shown by Maurer, Jester, and collaborators (Jester, Petroll et al. 1998; Jester, Li et al. 2001; Maurer, Parker et al. 2002) to relate directly to the degree and duration of ocular injury (Figure 2.1.3.b). Their work has shown the relationship between cell initial killing and the resulting irritation. In the cytotoxicity assays with monolayer cells, a similar relationship between potency and effective concentration is expected for killing 50% of the target cell population (Harbell, Koontz et al. 1997).

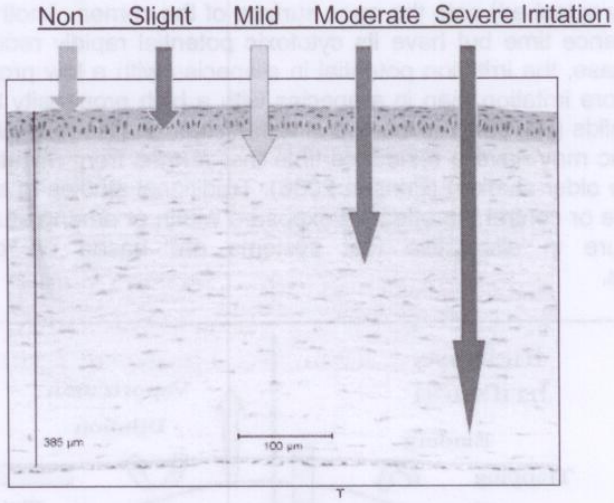


Figure 2.1.3.b Summary of the Depth of Injury Model

The CM estimates the metabolic rate (glucose utilization rate) of a population of cells by measuring the rate of excretion of acid by-products and resulting decrease in pH of the surrounding medium in an enclosed chamber. The rate of change in pH per unit time becomes the metabolic rate of the population. The basal metabolic rate and the ratio of glycolytic to aerobic metabolism (Krebs Cycle) may be different for different cell types. However, for the population of any one cell type, the ratio remains similar if the cells are handled in a consistent fashion. If a test material causes cytotoxicity to this population of cells it is assumed that the metabolic rate will fall. However, the metabolic rate may not fall immediately after exposure of the cells to a dilute concentration of toxicant. Populations of cells in culture are reported to metabolize glucose at only a fraction of their maximal metabolic rate (McConnell, Owicki et al. 1992). Thus, an up regulation of glucose metabolism can occur if the cells need energy to maintain their integrity in the face of a

mild biochemical insult. For example, exposure to a subcytotoxic concentration of surfactant can increase membrane leakage (to ions and water). This in turn can lead to an increase in the activity of ATP-dependent ion pumps and increased glucose metabolism. Thus early points in a killing curve can show increases in metabolic rate of 2- to 3-fold, but this metabolic rate then soon falls below 100% as higher concentrations of test material overwhelm the homeostatic controls within the cells (Figure 2.1.3.c).

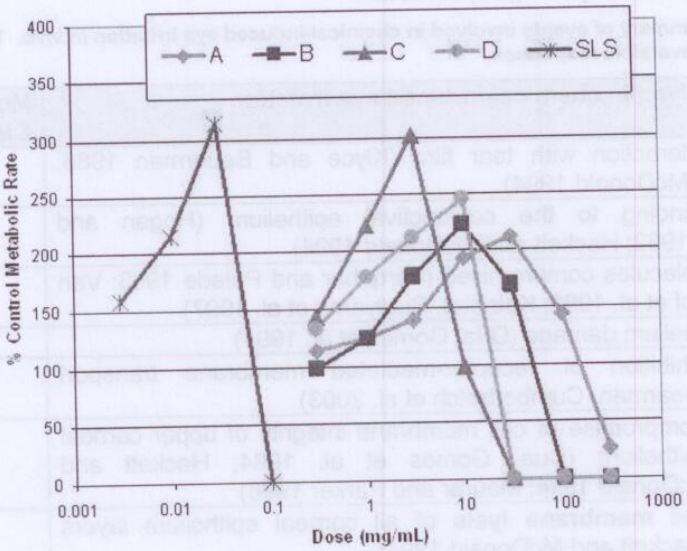


Figure 2.1.3.c Example of the metabolic rate data as a function of surfactant type and concentration

Although the metabolic rate is the physical parameter which is measured during the CM assay, the magnitude of metabolic rate itself is not directly related to eye irritation potential. Rather, the reduction of the metabolic rate to 50% of its basal rate is the parameter used to measure the impact of the test article on the test system (L929 cells in almost all cases). The CM assay exposes a population of cells to increasing concentrations of the test article (diluted in medium). The exposure follows a three step process where the first step is the exposure to the diluted test article, the second is the test article rinse-out and the third is the measurement of the metabolic activity. This means that the impact of the exposure is measured immediately and then a subsequent exposure is performed until the highest testable concentration has been used or the population of cells is severely damaged and the metabolic rate has declined to effectively zero. From the concentration response curve, the concentration that leads to a 50% decline in the metabolic rate of the population (the MRD_{50}) is calculated from the curve. The MRD_{50} values are used to compare test materials and provide a measure of ocular irritancy potential.

By current convention, the units of the MRD_{50} are mg/mL; however, many of the studies reviewed in this BRD presented data using related terminology, e.g. MRD_{50}

expressed in µg/ml or as the reciprocal of the MRD_{50}. For consistency we have converted all such values to MRD_{50} (mg/mL) and report them as such in this BRD.

For ease in understanding the mechanistic basis of the CM assay, a table (Table 2.1.3) has been compiled describing the events that are commonly considered to occur during eye irritation. Those events that are modeled (or are closely related) by the CM assay are indicated by a Y (yes) indication.

Table 2.1.3 Summary of events involved in chemical-induced eye irritation *in vivo*. Text in italics represents irreversible responses.

Events involved in chemical-induced eye irritation	Modeled by the CM assay?
Chemical interaction with tear film (Klyce and Beuerman 1988; Hackett and McDonald 1994)	N
Chemical binding to the conjunctival epithelium (Hogan and Zimmerman 1962; Hackett and McDonald 1994)	Y
Adhesion molecules compromised (Farquhar and Palade 1963; Van Meer, van Hof et al. 1992; Katahira, Sugiyama et al. 1997)	N
Corneal epithelium damage (Dua, Gomes et al. 1994)	Y
• Inhibition of receptor-mediated membrane transport (Dearman, Cumberbatch et al. 2003)	Y
• Compromise of cell membrane integrity of upper corneal epithelium (Dua, Gomes et al. 1994; Hackett and McDonald 1994; Maurer and Parker 1996)	Y
• Cell **membrane lysis** of all corneal epithelium layers (Hackett and McDonald 1994)	Y
Hydration of corneal stroma (Hackett and McDonald 1994)	N
Cross-linking *of proteins in corneal stroma (Butler and Hammond 1980; Eurell, Sinn et al. 1991; Chan and Hayes 1994)*	N
Erosion of corneal stroma (Baldwin, McDonald et al. 1973; Hackett and McDonald 1994; Maurer and Parker 1996)	N
Cell damage to corneal epithelium and limbus (Jacobs and Martens 1990; Wilhelmus 2001)	Partially
Dilation and increased lymphatic leakage from scleral vasculature (Hackett and McDonald 1994)	N
Stimulation of nerve endings, i.e. enhanced blinking, tearing (Chan and Hayes 1994)	N
Erosion of nerve endings in cornea and sclera (Butler and Hammond 1980; Klyce and Beuerman 1988; Araki, Ohahsi et al. 1994)	N
Duration of response, i.e. length of time cell responses deteriorate. Duration of response covers the effects of **reactive chemicals** which can cause **coagulation, saponification**, that are effects which develop and increase over time. (Hubert 1992; Maurer and Parker 1996)	N
Recovery from response, i.e. length of time for cell responses to return to control levels (Hubert 1992)	N

It can be seen that the CM assay most closely models some of the initial stages of interaction of an eye irritant with the cornea. The more distal occurrences in eye irritation such as gross tissue changes in the corneal stroma, and the recovery from the lesions, are not directly modeled. However, if the hypothesis of Jester, Mauer, and others that initial area and depth of injury is predictive of time to, and extent of, recovery, then the measurements made by the CM may have a relationship to recovery as well.

2.1.4 Similarities and differences of modes of action in the test method and the reference species

In the *in vivo* rabbit eye test, the depth of damage to the tissue through subjective observations of tissue changes is being indirectly assessed. One might also postulate that a test substance is progressively diluted with time in the eye (tearing and blinking) and with increasing depth of penetration (in part by binding with the lipids themselves). A more potent surfactant irritant will penetrate (kill progressive layers of cells) more readily because its effective exposure (i.e., the concentration sufficient to lyse cells) will be maintained into the deeper layers (since a lower concentration of a potent surfactant will still effectively lyse cells). *In vitro*, the test substance is first diluted and then each dilution is assessed for its ability to kill the target population. The minimum concentration of test material capable of damaging a given fraction of the target cells is being determined. In some assays (e.g., neutral red release), each dilution is tested with a separate population of cells while in the Cytosensor, the cell population is exposed progressively to each increasing concentration. The test system (target cell population) is very uniform (cell number and distribution) and the period of exposure tightly controlled. Thus, it is possible to determine the concentration required to damage a given fraction of the cells and use that value to compare the "potency" of surfactant to that test system.

In vitro assays for eye irritation fall into three general categories based on the dynamic range of the test system and assay endpoint. Assays involving cells in monolayer or suspension culture generally have a relatively small dynamic range (range between all alive and all dead) since all the cells in the system are exposed at exactly the same time to exactly the same concentration of test material. Therefore, the test article is diluted over a series of concentrations and applied to one or more cultures of the chosen cell type for a set exposure period (dilution-based assays) in order to more easily differentiate between the toxicities of various test materials. The fluorescein leakage (transepithelial passage), neutral red uptake, neutral red release, CM, and red blood cell lysis assays are examples of dilution-based assays. In every case, the endpoint value that is used to predict ocular irritation potential is the concentration of test article that produces a measured change in the test system (e.g., concentration required to reduce the treated cell population viability to 50% of the negative control cell population viability).

Besides the general concepts mentioned above, the following background information should be considered in the overall design of any CM study:

Since the CM is a dilution-based assay, some common strengths and weaknesses of all dilution-based assays should be considered. Among the strengths of dilution-based assays are: 1) the ability to carefully control the test system (cultured cells), 2) a wide assay dynamic range (range of dilutions), 3) the possibility for machine scoring of the endpoint (often spectrophotometric or fluorometric readings), 4) generally good intra-assay consistency, and 5) a relatively low cost.

Among the potential weaknesses of dilution-based assays are: 1) the need to dilute the test article in a physiological aqueous medium, 2) the difficulty in modeling deep tissue penetration, and 3) the immediacy of the endpoint assessment (lack of time for delayed responses to be manifested).

Certain types of test articles are poor candidates for testing in dilution-based assays. Hydrophobic chemicals or formulations (creams, pastes, or lotions) may never really reach the test system. Use of intermediate solvents would change the normal distribution of the test substance and they are generally not used. Organic solvents would be subject to dilution in aqueous medium so that the test system would not necessarily be exposed to the delipidizing or dehydrating effect of the solvent. Acids and alkali materials would be partially or fully neutralized. In all cases, these effects of dilution would serve to alter the test material and potentially impact the prediction of irritation. In contrast, surfactants and surfactant formulations appear to be less impacted by dilution in aqueous medium, as long as there is the recognition that the critical micelle concentration (CMC) is important. Surfactants form micelles at higher concentrations which reduces the number of surfactant molecules available to react with the target tissue. If the in-use concentration of the surfactant is below the CMC and the *in vitro* test is conducted at dilutions above the CMC, then a possible underestimation of the toxicity of the material could occur since cytotoxicity would be reduced at the concentrations above the CMC. Reduction of the Draize score as higher concentrations of pure surfactants are placed in the rabbit eye is commonly seen when dose-response experiments are conducted *in vivo* (Dr. Edward Bueller, Hilltop Research, Personal Communication)

2.2 Test method protocols

Table 2.2 Major components of the protocols

Table 2.2.a Major components of the protocols

		(Bruner, Miller et al. 1991)	(Bruner, Kain et al. 1991)	Corporate Company # 2 (Catroux, Rougier et al. 1993))	Corporate (Company # 3, positive control data)
Critical protocol elements	Instrument	Silicon microphys-iometer	Silicon microphys-iometer	Silicon microphys-iometer	Cytosensor
	Cells	NHEK	NHEK	L929	L929
	Cell #/Confluency	90-95%	$1x10^3$/100nL vol	$3x10^5$/well	$6x10^5$/well
	Coverslip/transwell	Coverslip	Coverslip	Transwell	Transwell
	Duration of exposure	320 second	~320 second	400 seconds	810 seconds
Positive controls		Unknown	Unknown	SLS	SLS*
Negative controls		Medium	Medium	Medium	Medium
Vehicle controls		NA	NA	NA	NA
Benchmarks used		None	None	Yes, but coded	NA
Endpoint(s) measured		MRD_{50}	MRD_{50}	MRD_{50}	MRD_{50}
Prediction Model(s) applied		None	None	None	95% Confidence interval
Quality control criteria used		Unknown	Unknown	Unknown	Performance of the positive control
GLP compliance		No	No	No	Generally yes
Availability of a standardized SOP		No	No	Summary data	Yes
Limits of Use Described		Not stated	Not stated	Not stated	NA
Reference data available		Summary	Summary	Summary	NA

* A 10% solution of SLS in DI H_20 is diluted in low-buffered medium ($NaCO_3$-free DMEM with additional NaCl and 1 mM sodium pyruvate supplemented with 2 mM L-glutamine and 50 µg/mL gentamicin). Final concentrations in one-half log does from 0.003 mg/mL to 3.0 mg/mL are dosed to determine an MRD_{50} with a historical average of 0.0798 mg/mL.

Table 2.2.b Major components of the protocols

		(Bagley, Bruner et al. 1992)	(Bagley, Bruner et al. 1992)	Corporate (Company # 1)	Corporate (Company # 1)
Critical protocol elements	Instrument	Silicon Microphys-iometer	Silicon Microphys-iometer	Silicon Microphys-iometer	Cytosensor
	Cells	L929	L929	L929	L929
	Cell #/Confluency	90-100%	$3x10^5$/well	90-100%	$6x10^5$/well
	Coverslip/transwell	Coverslip	Transwell	Coverslip	Transwell
	Duration of exposure	500 second	500 second	500 seconds	810 seconds
Positive controls		SLS	SLS	SLS	SLS
Negative controls		Medium	Medium	Medium	Medium
Vehicle controls		NA	NA	NA	NA
Benchmarks used		None	None	Yes but coded	Yes but coded
Endpoint(s) measured		MRD_{50}	MRD_{50}	MRD_{50}	MRD_{50}
Prediction Model(s) applied		None	None	Corporate	Corporate
Quality control criteria used		Unknown	Unknown	Performance of the positive control	Performance of the positive control
GLP compliance		No	No	Generally yes	Generally yes
Availability of a standardized SOP		No	No	Yes	Yes
Limits of Use Described		Not stated	Not stated	Generally surfactant-based materials	Generally surfactant-based materials
Reference data available		Summary	Summary	Full	Full

Table 2.2.c Major components of the protocols

		CTFA Phase III (Gettings, Lordo et al. 1996)	EC/HO (Balls, Botham et al. 1995)	COLIPA (Brantom, Bruner et al. 1997; Harbell, Osborne et al. 1999)	INVITTOX (IP-97)	INVITTOX (IP-102)
Critical protocol elements	Instrument	Silicon microphysiometer	Cytosensor	Cytosensor	Silicon microphysiometer	Cytosensor
	Cells	L929	L929	L929	L929	L929
	Cell #/Confluency	Confluent	~6x10^5/well	~6x10^5/well	90-100% confluency	5-6x10^5/well
	Coverslip/transwell	Coverslip	Transwell	Transwell	Coverslip	Transwell
	Duration of exposure	500 second	810 seconds	810 seconds	500 seconds	300 second
Positive controls		SLS	SLS	SLS	Unknown	None
Negative controls		Medium	Medium	Medium	Medium	Medium
Vehicle controls		NA	NA	NA	NA	NA
Benchmarks used		None	None	NA	None	None
Endpoint(s) measured		MRD_{50}	MRD_{50}	MRD_{50}	MRD_{50}	MRD_{50}
Prediction Model(s) applied		None	None	Yes	None	None
Quality control criteria used		Performance of the positive control	Performance of the positive control	Performance of the positive control; Audit of data by BIBRA QC Unit	Performance of the positive control	Unknown
GLP compliance		No	No	No	No	No
Availability of a standardized SOP		Yes	Yes	MA yes	Yes	Yes
Limits of Use Described		Surfactants only	Solubility in medium	Solubility in medium	pH ≤ 2.0 or pH ≥1 2.0	Solubility in medium
Reference data available		Yes (full data)	Yes (full data)	Yes (full data)		

2.2.1 Description of protocol components and rationale for differences

Overview of the methodology: The Cytosensor uses a low volume flow through chamber and a light-addressable potentiometer to measure the metabolic rate of a cell population. Metabolic rate is determined indirectly by the number of protons excreted into the low buffer medium (change in pH) per unit time. The light-addressable potentiometer forms the bottom of the flow through chamber and serves as a very sensitive and stable pH meter. While medium is flowing through the chamber, the pH is stable and governed by the medium. When the flow of medium is stopped, the pH begins to drop in a linear fashion over time. The actual change in pH during this measurement is generally less than 0.2 pH units.

Three common protocols developed by The Company # 1 were used in most of the studies reported in this BRD. The first two were based on the silicon microphysiometer (prototype instrument) and the third on the Cytosensor (commercial instrument). Most of the data in this BRD come from the third protocol. The two instruments differ primarily in the way the cells are introduced into the sensor chamber. The sensor chamber is composed of the light-addressable potentiometer sensor (sensor chip) on the bottom and ports for the medium (inlet and outlet). For the silicon microphysiometer, the cells were plated on metal coated glass coverslips and allowed to grow to confluence. The coverslip became the upper part of the low volume chamber with the cells on the downward side (facing the sensor chip). Medium then flowed between the coverslip and the sensor chip (Figures 2.2.1.a and 2.2.1.b). For the Cytosensor (and late modifications of the silicon microphysiometer), the cells are grown on a transwell membrane (discussed below). The whole transwell is placed into the sensor chamber and a plunger (with a spacer) pressed down on the membrane to seal it. There is a small medium-filled space between the sensor chip and the bottom of the transwell. The cells are attached to the top of the membrane so that the acid metabolites must pass through the membrane pores to reach the space in the lower part of the chamber. The medium is passed over the cells on the upper side of the membrane. The change from the coverslip system to the transwell system brought a change in the exposure, rinse, and read cycle as well. Figure 2.2.1.c shows the operating components of the instrument and Figure 2.2.1.d shows the low volume sensor chamber (transwell configuration). Based on the comparison of data generated in both the SM and CM, Company # 1 established a conversion algorithm so that all results generated initially from the SM can be compared to the results generated for the CM.

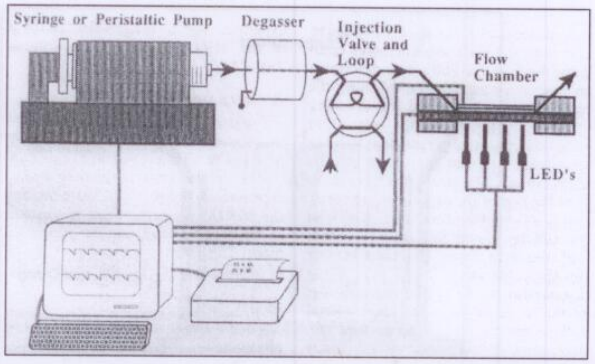


Figure 2.2.1.a Diagram of the operating components of the silicon microphysiometer (Bruner, Miller et al. 1991)

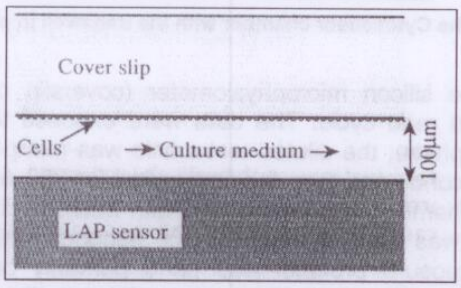


Figure 2.2.1.b The original silicon microphysiometer sensor chamber with the coverslip in place (Bruner, Miller et al. 1991)

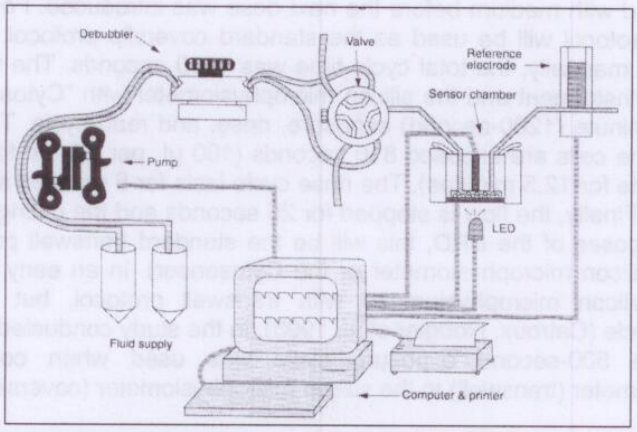


Figure 2.2.1.c Diagram of the operating components of the Cytosensor (Cytosensor Manual)

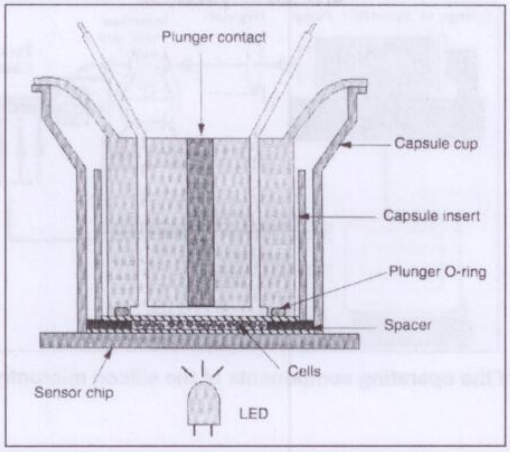


Figure 2.2.1.d The Cytosensor chamber with the transwell in place (Cytosensor Manual)

Originally, the silicon microphysiometer (coverslip chamber) used a 15-minute exposure, rinse, and read cycle. The cells were exposed to each concentration in two phases. In the first phase, the diluted test article was pumped (1.67 µL/sec) through the chamber for 120 seconds and then the flow halted for 200 seconds (total of 320 seconds of exposure). The chamber was then rinsed with fresh medium at the same rate for 380 seconds. The flow was then stopped for 200 seconds while the acidification rate was measured. This exposure protocol was used primarily on normal human epidermal keratinocytes (Bruner, Miller et al. 1991). Most of the studies in this BRD used L929 cells as the test system. The exposure protocol was altered so that the cells were exposed to the test article for a total of 500 seconds (300 seconds of flow and 200 seconds with the flow off), rinsed for 400 seconds, and the metabolic rate determined for 169 seconds. Flow was restarted with medium before the next dose was introduced. For the purposes of the BRD, this protocol will be used as the standard coverslip protocol. Because the valves were turned manually, the total cycle time was 1100 seconds. The Cytosensor (both the commercial instrument and the silicon microphysiometer with "Cytosensor-like" chambers used a 20-minute (1200-second) exposure, rinse, and read cycle. This is still the current protocol. The cells are exposed 810 seconds (100 µL per minute for one minute and 20 µL per minute for 12.5 minutes). The rinse cycle lasts for 6 minutes and the flow is 100 µL per minute. Finally, the flow is stopped for 25 seconds and the change in pH is measured. For the purposes of the BRD, this will be the standard transwell protocol (for either the converted silicon microphysiometer or the Cytosensor). In an early study, Company # 2 used the silicon microphysiometer with transwell protocol, but used a 400-second exposure cycle (Catroux, Rougier et al. 1993). In the study conducted by Bagley, Bruner et al. 1992 a 500-second exposure cycle was used when comparing the silicon microphysiometer (transwell) to the silicon microphysiometer (coverslip).

The bulk of the available data come from the transwell protocol using the 810-second exposure. These studies include the EC/HO, COLIPA, a large portion of the Company # 1 corporate database, and the positive control database from Company # 3.

The transwell was introduced by Company # 6 to allow more efficient introduction of the test system to the sensor chambers (including non-adherent cells in a gelatin matrix). However, this change limited the cell density and types of cells that could be used. The transwells have 3 micron pores that allow efficient communication between the upper surface of the membrane (with the cells) and the lower surface that faces the sensor itself. Confluent cell layers would interfere with this communication and so the cell density was reduced to a standard $6x10^5$ cells per well (seeded the day before use). The transwell uses a polycarbonate filter membrane that is less prone to interaction with test materials than other types of membranes but does not allow the human keratinocytes to attach. Thus, the L929 cells were selected because they would readily attach and were easy to grow in continuous culture. With the change to L929 cells, the SM exposure protocol was changed to 500 seconds. This is the protocol that was used in the Bagley, Bruner et al. 1992, and the Company # 1 corporate database. L929 cells were seeded to produce a confluent (or nearly confluent) cell layer on the coated coverslip. With the advent of the transwell sensor chamber, the 810-second exposure protocol was developed and this protocol was used in the EC/HO, COLIPA, the Company # 1 corporate database, and the Company # 3 positive control database.

2.2.1.1 Development of Conversion Algorithm between SM and CM

At the time that the SM was replaced with the CM by Company # 6, the Company # 1 sponsored a study to compare data obtained with the SM (coverslip protocol) for a set of 11 surfactant-containing materials with data obtained for the same materials with the CM (transwell protocol). The studies were carried out at a single laboratory (Company # 4). The raw data can be found in Annex F35. The testing protocol utilized a preliminary trial followed by at least three definitive trials. Data produced by the SM and CM are shown in Tables 2.2.1.1.a & b, respectively. It can be seen that the overall mean CV for each of the two methods is very similar (22.8% for the SM; 21.8% for the CM).

Following data collection from both instruments, the data were compared and the following equation was derived to translate SM coverslip data to CM transwell data:

$$\text{Log}_{10}\,(\text{Cytosensor MRD}_{50}) = 0.135 + 0.7753 \times \text{Log}_{10}\,(\text{Silicon Microphysiometer MRD}_{50}).$$

A graph depicting the relationship between the SM and CM is given in Figure 2.2.1.1. The current standard Cytosensor protocol is attached in Annex A. Other protocols, including the INVITTOX protocols 97 and 102, are included in Annex A.

Table 2.2.1.1.a Silicon Microphysiometer data for 11 surfactant-containing materials from Company # 1 (See Annex F35)

Substance	Prelim*	Trial 1	Trial 2	Trial 3	Trial 4	Mean MRD_{50} (mg/mL)	SD	N	CV (%)
A	21.368	18.116	25.510	20.408		21.345	3.785	3	17.7
B	+	0.083	0.085	0.082		0.083	0.001	3	1.7
C	+	0.291	0.266	0.263		0.273	0.015	3	5.5
D	+	0.247	0.153	0.435	0.298	0.283	0.117	4	41.5
E	+	13.643	13.004	9.434		12.027	2.268	3	18.9
F	+	0.042	0.027	0.026		0.032	0.009	3	28.2
G	0.161	0.093	0.139	0.198		0.143	0.053	3	36.8
H	0.714	2.020	1.239	1.595		1.618	0.391	3	24.2
I	0.094	0.043	0.032	0.039		0.038	0.006	3	14.7
J	0.020	0.045	0.038	0.026		0.036	0.010	3	26.9
K	+	0.081	0.094	0.152		0.109	0.038	3	34.5
Mean									**22.8**
Median									**24.2**

* Not included in the mean calculation
\+ Value not determined during assay

Table 2.2.1.1.b Cytosensor Microphysiometer data for 11 surfactant-containing materials from Company # 1 (See Annex F35)

Substance	Prelim*	Trial 1	Trial 2	Trial 3	Trial 4	Mean MRD_{50} (mg/mL)	SD	N	CV (%)
A	90.909	56.497	48.544	62.500		55.847	7.001	3	12.5
B	0.223	0.254	0.424	0.283		0.320	0.091	3	28.4
C	0.758	0.794	0.552	0.820		0.722	0.147	3	20.4
D	0.452	0.442	0.412	0.431		0.428	0.016	3	3.7
E	19.120	9.091	11.429	5.319		8.613	3.083	3	35.8
F	0.067	0.074	0.052	0.075		0.067	0.013	3	19.2
G	0.251	0.177	0.288	0.267		0.244	0.059	3	24.3
H	2.288	2.110	2.016	2.457		2.194	0.232	3	10.6
I	3.497	1.475	4.367	3.802		3.215	1.533	3	47.7
J	0.282	+	0.139	0.151	0.165	0.152	0.013	3	8.5
K	0.251	0.268	0.159	0.281		0.236	0.067	3	28.4
Mean									**21.8**
Median									**20.4**

* Not included in the mean calculation
\+ Value not determined during assay

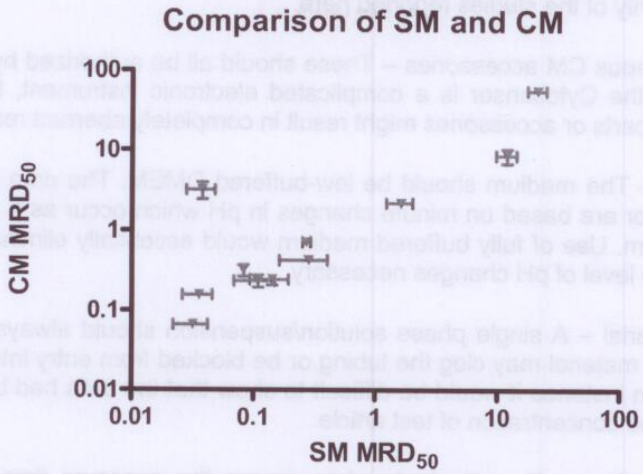


Figure 2.2.1.1 A comparison of data obtained from 11 surfactant-containing products with SM and CM.

2.2.2 Proposed critical components of the protocol that impact on reproducibility and/or predictive capacity of the assay

Because of the somewhat intricate nature of Cytosensor studies (due in a large part to the electro/mechanical complexity of the instrument), the initiator of any study conducted on the current CM machine according to protocols similar to the current protocol of Company # 3 (Annex A3) or INVITOX 97 or 102 (Annex A25 & A35) should be aware of the following Critical Protocol steps:

- Target Cells - The cell line of choice for ocular irritation assays is L929 cells. Although data from the use of other cell types, e.g. normal human keratinocytes, has been reported, virtually all safety studies conducted with the Cytosensor instrument (which uses a transwelll chamber rather than a coverslip) since the early 1990's have used L929 cells. Use of normal human keratinocytes with the transwell is not recommended.

- Instrument – The Cytosensor Microphysiometer manufactured by Company # 6, is the only instrument we are aware of that will produce the same type of data as are reported in this BRD. Results obtained with a predecessor instrument, the Silicon Microphysiometer, are contained in the BRD; however, the main prediction model described is only based on Cytosensor data.

- Capsule insert – This item must not be used in the assay. Although described in the user information which accompanies the Cytosensor, it has not been used for the vast majority of the studies reported here.

- Miscellaneous CM accessories – These should all be authorized by Company # 6. Because the Cytosensor is a complicated electronic instrument, the use of non-standard parts or accessories might result in completely aberrant readings.

- Medium – The medium should be low-buffered DMEM. The data provided by the Cytosensor are based on minute changes in pH which occur as a result of cellular metabolism. Use of fully buffered medium would essentially eliminate the ability to detect the level of pH changes necessary.

- Test material – A single phase solution/suspension should always be used. Non-dissolved material may clog the tubing or be blocked from entry into the chambers. In such an instance it would be difficult to show that the cells had been exposed to the desired concentration of test article.

- Exposure time – For standard safety assays the exposure time should be 810 seconds in order to match the experimental conditions for which the main prediction model was established. Longer or shorted exposure times will change the calculated MRD_{50} since toxicity is a function of exposure time.

- Baseline rates – It must always be ascertained that the machine and cells are stable before the experiment can begin because all subsequent data points are interpreted based on the baseline rate. In general this baseline should be between 50 and 200 microvolts/sec for 4 – 5 readings.

- Positive control – A positive control must be established in the user's laboratory so that there is assurance that Cytosensor is giving similar readings from day-to-day. Without this control it is impossible to compare the data for different test articles tested on different days.

- Cell density – Cells should be seeded at a density of ~6 X 10^5 cells/cup and then incubated for 16 – 32 hours under normal growth conditions before use. At the time of use the cells should be <80% confluent. Use of a fully confluent monolayer may interfere with communication between the upper and lower surfaces of the membrane, causing inaccurate readings.

- Confirmatory assay – It is strongly suggested that a confirmatory assay should always be conducted. Because the Cytosensor is an extremely sensitive instrument with a number of chambers, it has the potential to give spurious readings for some chambers which could skew the results. These anomalies might not be reflected in the performance of the positive control. Although this is rare, it is prudent to always confirm the response before reaching a safety conclusion.

2.2.3 List of studies with similar protocols

The Bagley, Bruner et al. 1992 study and the unpublished Silicon Microphysiometer study from Company # 1 were conducted with similar protocols. In Table 2.2.3.a, the critical protocol elements are listed. Company # 1 generally conducted their study according to GLP practices and with a positive control which was a quality control criterion for a valid test. Although Bagley, Bruner et al. 1992 did not conduct their study according to GLPs, the critical protocol elements remain the same as the Company # 1 study.

Table 2.2.3.a Silicon Microphysiometer studies with similar protocols

		Bagley, Bruner et al. 1992	Corporate (Company # 1)
Critical protocol elements	Instrument	Silicon Microphysiometer	Silicon Microphysiometer
	Cells	L929	L929
	Cell #/Confluency	90-100%	90-100%
	Coverslip/transwell	Coverslip	Coverslip
	Duration of exposure	500 second	500 seconds

The COLIPA (Brantom, Bruner et al. 1997), unpublished Cytosensor Microphysiometer study from Company # 1, positive control data from Company # 3, and EC/HO were conducted with similar protocols. In Table 2.2.3.b, the critical protocol elements are listed.

Table 2.2.3.b Cytosensor Microphysiometer studies with similar protocols

		COLIPA (Brantom, Bruner et al. 1997)	Corporate (Company # 1)	Corporate (Company # 3, positive control data)	EC/HO (Balls, Botham et al. 1995)
Critical protocol elements	Instrument	Cytosensor	Cytosensor	Cytosensor	Cytosensor
	Cells	L929	L929	L929	L929
	Cell #/Confluency	$6x10^5$/well	$6x10^5$/well	$6x10^5$/well	~$6x10^5$/well
	Coverslip/transwell	Transwell	Transwell	Transwell	Transwell
	Duration of exposure	810 seconds	810 seconds	810 seconds	810 seconds

2.2.4 Known applicability and limitations of the assay

The Cytosensor microphysiometer (CM) and its predecessor instrument the silicon microphysiometer (SM) have been in use with various toxicology protocols for over 15 years. Prediction of eye irritation was one of the first proposed uses (Parce, Owicki et al. 1989) and initial studies were conducted with a range of chemicals, e.g. solvents,

surfactants, alcohols, etc. As with any new technology, a range of chemical classes were evaluated to determine the applicability domain(s) and strengths and limitations of the assay (physical form, extremes of pH, etc). However, early on the major focus began to be placed on liquid soaps, detergent formulations, and household cleaning products (Bruner, Miller et al. 1991). Because of physical restrictions (the test material must be pumped into a chamber containing the test cells and then completely removed in the same fashion), the applicability domain has generally been considered restricted to test materials that are completely aqueous soluble. Currently, the assay is used primarily for evaluating the eye irritation potential of liquid surfactant-containing formulations/mixtures.

Because of the unique characteristics of the SM or CM instruments, the applicability domain is immediately limited to testing fully water soluble materials. The instrument functions by pumping the test material through a very small diameter hose onto the cells in the transwell. Any particulate matter that is present in the dosing solution could either clog the hoses - immediately ending the experiment - or might settle out on the transwell or on the cells themselves making it difficult, if not impossible, to determine the actual dose to which they were exposed. Table 2.2.4 describes the physiochemical properties of test materials and their compatibility with the CM assay for eye irritation.

Table 2.2.4 Physicochemical properties of test materials and their compatibility with the CM assay for eye irritation

Physicochemical Property	Is a material with this property compatible with the CM assay system? (based on solubility)
Fixative	No
Solvent	Yes, if aqueous soluble some solvents are testable
Extreme pH	No
Gases	No
Liquids	Yes, but must be aqueous soluble
Solid materials	Yes, if aqueous soluble, but cannot be tested in its solid form
Emulsions	No
Granular materials	No
Suspensions	No
Coloured materials	Yes
Toxicity affected by dilution	No
Highly viscous materials	No
Volatile materials	No
Reactive chemistries	No
Hydrophobic/lipophilic chemicals	No
Neat concentrations of chemicals	Yes, but a serial dilution will be performed with the neat concentration being the last dilution tested

2.2.5 Proposed prediction models

Historically, the only published prediction model for the CM is the one utilized in the COLIPA validation study ((Brantom, Bruner et al. 1997; Harbell, Osborne et al. 1999) which relates the MRD_{50} to the Draize Modified Maximum Average Score (MMAS) for

surfactant and surfactant-containing compounds. Expressed as an equation, the relationship is:

$$MMAS = A/1 + e^{B*(\log10MRD50 - G)}$$

where A = 148.0, B = 1.813 and G = 2.329. This relationship was developed using information from the testing of 133 materials.

However, during the development of the current ECVAM-sponsored BRD, the management team requested that proposed prediction models based on classification criteria that are described by the Global Harmonized System, the EU classification system and the USEPA classification system be submitted prior to the final analysis of the predictive capacity of the data in the BRD.

Consequently we submitted three classification prediction models. The only existing PM that we were aware of that addressed categorical labeling was one under development for the USEPA as part of a project to develop non-animal labeling methods for anti-microbial cleaning products. That preliminary PM was:

MRD_{50} < 3 min = EPA I
80 min > MRD_{50} > 3 min = EPA III
MRD_{50} > 80 min = EPA IV

There were not sufficient data available to define a cut-off for EPA II materials, so by default these materials were included in the EPA I prediction interval and therefore overpredicted by one category.

The preliminary PM's for the GHS and EU system which were submitted were hypothesized using only a very early analysis of data and with knowledge of the only slightly better developed USEPA PM.

For the EU system the proposed PM was:

	MRD_{50}
R41	<3 min
R36	<10 min; >3 min
Not Classified	>10 min

For the GHS system the proposed PM was:

	MRD_{50}
1	<3 min
2A or 2B	<80 min; >3 min
No Category	>80 min

Following more detailed data analysis in the finalization of this BRD, *post hoc* evaluation of the data led to the following PM's on which the subsequent analysis of the predictive capacity of the CM assay for surfactants and surfactant containing formulations was based.

For the EU system the proposed PM is:

	MRD_{50}
R41	<2 min
R36	<10 min; >2 min
Not Classified	>10 min

For the GHS system the proposed PM was:

	MRD_{50}
1	<2 min
2A or 2B	<10 min; >2 min
No Label	>10 min

For the USEPA system the proposed PM was:

	MRD_{50}
I	<2 min
III	<80 min; >2 min
IV	>80 min

It can be seen that only slight modifications occurred when a more data were considered in the analysis. For all three classification systems the cut-off for the most severe labeling category was increased from <3 minutes to <2 minutes. This minor change appeared to fit the data distribution better without sacrificing sensitivity for the severe materials.

A second change was making the cut-off for GHS No Label materials >10 minutes rather than >80 minutes. This change was necessitated because we based our originally proposed classification on the data that had been developed for the USEPA classification work. However many materials which are EPA Category III materials fall into the lower GHS category (No Label) when evaluated by the GHS criteria. Thus the cut-off for the GHS No Label could be lowered to the less stringent >10 minutes.

It should be noted that the data analyzed to construct all three prediction models had fewer materials in the intermediate labeling categories (GHS 2A and 2B, EU R36, and EPA II and III) than in the severe or mild categories. In fact insufficient data were available to differentiate the two intermediate categories for either the GHS or USEPA system.

3. Within-laboratory reproducibility (Module 2)

The structure of almost all protocols analyzed in this BRD requires that SM or CM results for individual test materials be determined by averaging the results of at least three separate runs, where each run consists of testing multiple test article doses on a single cell culture. Replicate runs generally take place on a single day with a single operator, although they can occur on separate days with separate operators. Therefore, within-laboratory reproducibility for the SM or CM can be of two types: reproducibility in replicate cultures for a single test material done in multiple runs, generally by the same operator, and on the same day or within a few days (Type 1); and reproducibility of mean results for single test materials done in separate experimental settings and separated by longer time periods, perhaps of several years (Type 2). In the following analysis, the type of reproducibility being studied will be indicated.

3.1 Within-laboratory reproducibility for studies where raw data are available

Table 3.1 lists the various studies for which within-laboratory reproducibility information was available and describes the experimental parameters for each of the studies.

Table 3.1 Table presenting the results and relevant information pertinent to within-laboratory reproducibility for studies where raw data are available.

Studies	Results Reported			Replicates			Format of data (raw data, summary results, other)
	Intralaboratory Variability	Instrument	Number of test substances	No. of operators	No. of experiments	No. of replicates/ experiment	
The Company 1[1] (Bruner, Miller et al. 1991)	Yes; Mean, SEM and number of replicates	SM	17 of 17 tested	Unknown	1	N = 3-11	Summary; Mean ± SEM
Company # 2[2] (Catroux, Rougier et al. 1993)	Yes; Mean and SD – converted here to CV.	SM	18 of 21 tested	Unknown	1	3	Summary; Mean ± SD
EC/HO[3] (Balls, Botham et al. 1995)	No variability info. reported but calculated from raw data for this BRD	CM	35 of 60 tested	Unknown	1	≥ 3	Summary; Mean
CTFA[4] (Gettings, Lordo et al. 1996)	Yes; Mean, graphed SEM, and no. of replicates	SM	24 of 25 tested	Unknown	1	3 (20 materials), 4 (5 materials)	Summary; Mean
COLIPA[5] (Brantom, Bruner et al. 1997; Harbell, Osborne et al. 1999)	No, but calculated for this BRD	CM	26 or 29 of 55 tested[6]	Unknown	1	≥ 3 for each of 2 labs	Raw data; Mean, SD and CV
Company # 4/Company # 3 positive control[5]	Not published	CM	1 of 1 tested	Multiple	629	1	Raw data; mean, SD and CV
20 chemicals from EC/HO and the COLIPA study run by same lab	No, but calculated for this BRD	CM	16 of 20 tested	Unknown	2	≥ 3	See above for individual studies
Company # 1/ Company # 3 comparison study for SM and CM	Results not published, but calculated for this BRD	SM & CM	11 of 11 tested	1	1	≥ 3	Raw data; Mean, SD & CV

[1] Performed using the silicon microphysiometer coverslip protocol with a 320-second exposure and normal human epidermal keratinocytes (see (Bruner, Miller et al. 1991)).
[2] Performed using the silicon microphysiometer transwell protocol with the 400-second exposure cycle and L929 cells.
[3] Performed using the Cytosensor transwell protocol with an 810-second exposure and L929 cells.
[4] Performed using the silicon microphysiometer coverslip protocol with a 500-second exposure and L929 cells.
[5] Performed using the Cytosensor transwell protocol with an 810-second exposure and L929 cells.
[6] One lab tested 26 materials while a second lab tested 29 materials.

3.2 Compilation of results

Eight studies or data sets (Table 3.1) were identified as providing information relevant to this analysis. Type 1 reproducibility (reproducibility of runs over a short time frame) information was obtained from seven data sets, although only summary information was obtained for the Bruner, Kain et al. 1991 study. Type 2 information (reproducibility of runs over long time frame) was obtained from the Company # 3 positive control data set which spanned over 12 years, and from a comparison between the mean results for 20 chemicals which were tested in both the EC/HO study and the COLIPA study. These two studies were conducted nearly two years apart.

3.2.1 Statistical approaches used: description & rationale for the approach used

Means, standard deviations (SD), and coefficients of variation (CV) were calculated (or in some cases transcribed directly from the manuscript) for the chemicals and formulations in all the studies except one (Bruner, Kain et al. 1991). For that study, the raw data could not be obtained, so only the mean and standard error of the mean (SEM) are reported as described in the manuscript.

CV values can sometimes be deceiving when used to analyze *in vitro* data which can range over several orders of magnitude, as is representative of the data in this BRD. Relatively small errors for small means can result in high CV's making the replicates appear to be variable. However, when the effect of these error ranges on the predicted value which may range over only two orders of magnitude (MAS for example) are examined, it can be seen that even an apparently large CV may have very little effect on the MAS value or hazard category that would be predicted.

3.2.2 Results and discussion

A presentation of the data and a discussion of the reproducibility it represents is contained in the following subsections. Where possible, the exact material tested is described to facilitate a more detailed analysis of the reasons for the lack of reproducibility where it occurs.

3.2.2.1 Company # 1study

The first Company #1 study reported (Bruner, Miller et al. 1991) compared a series of internal SM runs (3-11 per material) for 17 materials. The data were reported as average MRD_{50}; however, no SEM or SD were available. The data were also reported as average $pMRD_{50}$ ± SEM which cannot be easily converted to MRD_{50} ± SEM. The $pMRD_{50}$ is the negative logarithm of the average MRD_{50} in g/mL. The CV for these materials ranges from 0.6% to 16.9% which is considered to be quite low; however, it is based on log values so it cannot be directly compared with non-transformed data. The CV's of MRD_{50}'s expressed in log values will always be lower than the CV's of MRD_{50}'s expressed in normal concentration values.

Table 3.2.2.1 Within-laboratory reproducibility of SM from Bruner, Miller et al. 1991 using human keratinocytes and a 320 second exposure

Substance	MRD_{50} (mg/mL)	$pMRD_{50}$*	SD[1]	CV (%)[1]	Number of replicates
Surfactant Chemicals					
BAC (10%)	0.59	3.23	0.16	4.9	5
Sodium dodecylsulphate (40%)	0.55	3.26	0.03	0.9	3
Triethanolamine	17	1.77	0.3	16.9	11
Tween 20	17	1.77	0.22	12.4	5
Mean – Surfactant Chemicals				8.8	
Median – Surfactant Chemicals				8.7	
Surfactant Formulations					
Bar Soap 1	12	1.92	0.07	3.6	3
Bar Soap 2	7.8	2.11	0.22	10.4	6
Fabric Cleaner	18	1.74	0.21	12.1	3
Hand Soap	2.1	2.68	0.22	8.3	5
Hard Surface Cleaner 1	3.2	2.49	0.18	7.2	5
Hard Surface Cleaner 2	5.7	2.24	0.03	1.3	3
Heavy Duty Dishwashing Liquid	0.25	3.55	0.15	4.2	6
Heavy Duty Laundry Detergent	0.17	3.78	0.16	4.2	5
Light-Duty Dishwashing Liquid	0.69	3.16	0.02	0.6	4
Shampoo 1	0.77	3.11	0.09	2.9	5
Shampoo 2	0.61	3.21	0.02	0.6	3
Shampoo 3	0.39	3.41	0.1	2.9	3
Shampoo 4	1	3	0.3	10	11
Mean – Surfactant Formulations				5.3	
Median – Surfactant Formulations				4.2	
Mean – All Materials				6.1	
Median – All Materials				4.2	

* $pMRD_{50}$ is the negative log of the MRD_{50} in g/mL
[1] The SD and CV are based on the $pMRD_{50}$ values

3.2.2.2 Company # 2 study

Company # 2 reported a 53 chemical and product study (Catroux, Rougier et al. 1993) using the SM and L929 cells with three replicates for each material. Means and SD were available for 20 of the 21 surfactants. However, two of the surfactants were very non-toxic giving an average MRD_{50} of greater than 100 mg/ml. Because some of the individual data points for these two materials were censored (i.e. reported as "less than" or "greater than" a specified value), no accurate CV could be calculated. Therefore, only data for 18 of the 20 materials are reported in Table 3.2.2.2. The standard deviations were supplied to us by the authors from Company # 2. We did not calculate the SD's, but we did calculate the CV's using the values fromCompany # 2. The CV's for the 18 materials ranged from 5.7% to 64.6%. The test materials were all pure surfactants or surfactant blends. The mean CV was 23.0%.

Table 3.2.2.2 Within-laboratory reproducibility from the Company # 2 study of Catroux, Rougier et al. 1993 which used the SM with a 400 sec exposure time. See Annex H3.

Substance	MRD_{50} (mg/ml)	SD	CV (%)	Number of replicates
Surfactant Chemicals				
1,2-dodecanol (etherified)	0.851	0.05	5.7	3
Acylamine polyglycol ethersulfate (genapol AMS)	5.357	1	18.7	3
Ammonium laurylsulphate	0.129	0.017	13.1	3
Blend of decanol and dodecanol (both etherified)	1.288	0.8	46.6	3
Blend of sodium and magnesium laurylethersulfate	0.871	0.15	17.6	3
Cocobetain derivative	0.263	0.17	64.6	3
Coprah amphoteric alky limidazolium dicarboxylate (miranol)	0.575	0.08	13.7	3
Dodecanol (etherified)	0.468	0.07	15.1	3
Hexadecyltrimethylammonium bromide (CTAB)	0.04	0.011	27.5	3
Industrial Tween 20	7.08	1.6	23.1	3
Octylphenoxy polyethoxy ethanol (Triton X100)	0.068	0.013	19.1	3
Polyoxyethylene sorbinate monolaurate (Tween 20)	20.89	7	34	3
Pyridinium cetyl bromide	0.105	0.02	19	3
Sodium lauryl sulphate (SDS)-A	0.079	0.022	27.8	3
Sodium laurylethersulphate	0.126	0.025	19.8	3
Sodium laurylsarcosinate	0.229	0.045	19.9	3
Tetradecyltrimethylammonium bromide (MTAB)	0.043	0.004	9.3	3
Triethanolamine laurylsulphate	0.056	0.011	19.7	3
Mean			**23.0**	
Median			**19.4**	

3.2.2.3 CTFA Phase III study

In 1992, CTFA began a study of the ability of a number of *in vitro* test methods to predict eye irritation potential of a series of surfactant-based personal care formulations. The SM was one method studied, and it was used in only one laboratory – Company # 4 Three replicates were conducted on each test material. The replicate assays may have occurred on the same day or within a matter of a few weeks at the most. All of the SM assays were conducted between 28 July 1992 and 17 September 1992 (see Annex F3) at Company # 4 the instrument used was the SM using L929 cells grown on a coverslip and a 300 second exposure time.

The results presented in Table 3.2.2.3.a show CV's ranging from 1.8% to 61.4% for the 25 products. There was no clear pattern as to which types of materials caused the greater variability. For example, four of the twelve shampoos (Shampoos 2, 5, 7, and 8) had four of the five lowest CV's, while two other shampoos (Shampoo 1 and Shampoo AntiD) had the two highest CV's. However, it was interesting to note that both of the more variable shampoos had the lowest water content (14% and 27%, respectively) of any of the 25 tested formulations. A graphical representation *in vitro* vs. *in vivo* data in Figure 3.2.2.3 showed that the SM CV's were of a similar magnitude to those of the *in vivo* test (Gettings, Lordo et al. 1996).

Data from multiple runs of the positive control (SLS starting concentration of 10% solution; Annex F2) conducted during this study provide another measure of within laboratory reproducibility. Table 3.2.2.3.b lists the eighteen results with the positive control (one positive control was run each day that a batch of test materials were run) over a nearly two month period. The CV for the 18 runs was 9.6%.

Table 3.2.2.3.a Within-laboratory reproducibility of SM from archived data originally obtained at Company # 4 and created for the CTFA Phase III study (Gettings, Lordo et al. 1996). The protocol utilized the SM and a 500 second exposure. Data from Annex F3.

Cytosensor Identification	Substance	Mean SM MRD_{50} (mg/mL)	SD	CV (%)	Number of replicates
		Surfactant Formulations			
PGB-1	Baby Shampoo 1	2.13	0.52	24.5	3
PGH-1	Baby Shampoo 2	1.13	0.41	36	3
PGC-1	Bubble bath	0.65	0.24	36.9	3
PGQ-1	Cleansing Gel	6.54	2.05	31.3	3
PGF-1	Eye Makeup remover	32	9.79	30.6	3
PGT-1	Facial Cleaner	>500	0	Not Calculated	3
PGD-1	Facial Cleansing Foam	6.19	2.75	44.4	4
PGS-1	Foam Bath	0.75	0.14	18.2	3
PGZ-1	Gel Cleanser	2.99	0.95	31.7	3
PGI-1	Hand Soap	5.13	1.89	36.9	4
PGR-1	Liquid Soap 1	2.53	0.07	2.8	3
PGX-1	Liquid Soap 2	2.34	0.38	16.2	3
PGU-1	Mild Shampoo	7.31	1.14	15.6	3
PGN-1	Polishing Scrub	55.8	24.2	43.3	4
PGV-1	Shampoo 1	1.35	0.8	59.4	4
PGK-1	Shampoo 2	0.85	0.05	5.4	3
PGP-1	Shampoo 3	2.89	0.74	25.5	3
PGO-1	Shampoo 4	2	0.74	36.9	3
PGM-1	Shampoo 5	2.51	0.05	1.8	3
PGG-1	Shampoo 6	2.25	0.38	17.1	3
PGL-1	Shampoo 7	0.83	0.02	2.3	3
PGE-1	Shampoo 8	2.53	0.1	4	3
PGJ-1	Shampoo AntiD	0.79	0.48	61.4	4
PGW-1	Shower Gel	0.79	0.07	9	3
PGY-1	Skin Cleaner	0.75	0.1	13.3	3
Mean				**25.2**	
Median				**25.0**	

Table 3.2.2.3.b Within-laboratory reproducibility of the positive control at Company # 4 during the CTFA Phase III study (Gettings, Lordo et al. 1996). Data from Annex F3.

Date	SM MRD_{50} (mg/mL)
7/28/92	0.0650
7/29/92	0.0802
8/3/92	0.0861
8/4/92	0.0869
8/5/92	0.0806
8/10/92	0.0746
8/11/92	0.0881
8/12/92	0.0736
8/17/92	0.0863
8/18/92	0.0906
8/19/92	0.0904
8/24/92	0.0803
8/25/92	0.0838
8/26/92	0.0857
9/14/92	0.0921
9/15/92	0.102
9/16/92	0.0856
9/17/92	0.0845
Mean	**0.0842**
Median	**0.0857**
SD	**0.0081**
CV (%)	**9.60**

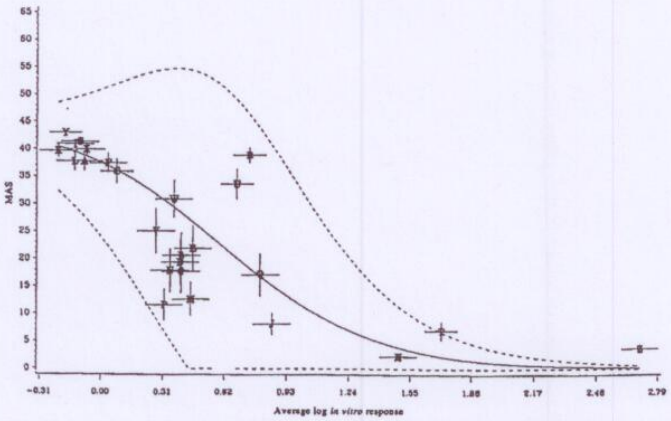


Figure 3.2.2.3 Regression plot of the relationship between *in vitro* endpoint and MAS for the SM; dashed curves represent 95% prediction bounds (Gettings, Lordo et al. 1996). Error bars represent one standard error.

3.2.2.4 EC/HO study

The EC/HO study of *in vitro* methods for eye irritation was designed to have three or more laboratories using each *in vitro* method. Four laboratories used the CM instrument with transwells and an 810 second exposure time. However, since only submission of mean CM values for each test material was requested by the Management Team, it has been difficult to obtain data from individual runs. At this point, raw individual run data for only 35 materials (the only ones determined by Company # 4 to be compatible with the CM) from one laboratory (Company # 4) have been located in the archives of the Institute For In Vitro Sciences. Only 32 of the 35 data points could be unequivocally linked to a specific test material. These data are presented in Table 3.2.2.4.

The raw data mean was compared against the published mean (SM31) to decode the individual raw data points (Annex G and H3). The mean MRD_{50} presented in the tables below was based on the average of transformed individual trial data ($rMRD_{50} \rightarrow MRD_{50}$), <u>not the transformation of the mean log MRD_{50} which is presented in subsequent tables in this report</u>. Values not used to calculate the reported mean are the preliminary (range finding) assay results which are used to narrow the dilution scheme and which in general practice is not used to calculate the mean. In addition, any other assays run (assays B - D) are assays in which an MRD_{50} could not be calculated because the cells did not die, or the assay was terminated early.

Table 3.2.2.4 Within-laboratory reproducibility of CM from archived data that was originally obtained at Company # 4 for the EC/HO study (Balls, Botham et al. 1995). The protocol utilized the CM using transwells and an 810 second exposure time. At least triplicate runs were performed. Raw data can be found in Annex H3. Surfactants are highlighted. N=35.

			Values used to calculate the reported Mean MRD_{50} (mg/mL)					Values not used to calculate the reported Mean MRD_{50} (mg/mL)					Results using the values used to calculate the reported mean				Results using all values other than > or < values to calculate mean[a]			
Sponsor Designation	Chemical	Formulation Type	Assay 1	Assay 2	Assay 3	Assay 4	Assay 5	Range Finding Assay	Assay A	Assay B	Assay C	Assay D	Average MRD_{50} (mg/mL)	N	SD	CV (%)	Average MRD_{50} (mg/mL)	N	SD	CV (%)
2376	2,5-Dimethylohexanediol	SO		151.36	151.36	165.96		>5.00					156.22	3	8.43	5.4	156.22	3	8.43	5.4
207	Acetone	SO		144.54	165.96	114.82		>5.00					141.77	3	25.68	18.1	141.77	3	25.68	18.1
2162	Ammonium nitrate	Other		169.82	125.89	144.54		>5.00					146.75	3	22.05	15.0	146.75	3	22.05	15.0
1373	Benzalkonium chloride 10%	SU			0.48	0.43	0.50	0.35	>3.50	<3.00			0.47	3	0.04	8.2	0.44	4	0.07	15.6
527	Benzalkonium chloride 5%	SU		0.95	0.98	1.38		3.01					1.10	3	0.24	21.7	1.58	4	0.97	61.5
3090	Benzalkonium chloride [1]/[2]	SU		5.01	3.89	7.08		3.02					5.33	3	1.62	30.4	4.75	4	1.75	36.9
1223	Cetylpyridinium bromide 10%	SU		0.98	1.15	0.93		3.09					1.02	3	0.11	11.1	1.54	4	1.04	67.6
1383	Cetylpyridinium bromide 5%	SU		1.26	0.72	2.69		1.20					1.56	3	1.02	65.3	1.47	4	0.85	57.8
2859	Cetylpyridinium bromide 0.1%	SU		128.82	91.20	91.20		>5.00					103.74	3	21.72	20.9	103.74	3	21.72	20.9
2653	Ethanol*	SO		162.18	67.61	147.91		>5.00					125.90	3	50.98	40.5	125.90	3	50.98	40.5
467	Ethyl acetate	SO				53.70		>4.70	>4.75	>4.75			53.70	1			53.70	1		
1719	Gammabutyrolactone	Other		93.33	144.54	112.20		>5.00					116.69	3	25.90	22.2	116.69	3	25.90	22.2
3111	Glycerol	SO		218.78		131.83	204.17	>5.00		>5.38			184.93	3	46.56	25.2	184.93	3	46.56	25.2
1666	Imidazole*	SU			23.44	22.39	23.44	24.55	>5.25				23.09	3	0.61	2.6	23.45	4	0.88	3.8
389	Isobutanol	SO		29.51	28.84	27.54		4.60					28.63	3	1.00	3.5	22.62	4	12.04	53.2
398	Isopropanol	SO		89.13	93.33	91.20		4.74					91.22	3	2.10	2.3	69.60	4	43.27	62.2
1581	L-Aspartic acid	AC		0.85	2.63	0.71		2.75					1.40	3	1.07	76.7	1.74	4	1.11	63.8
372	Methyl acetate	SO		89.13	93.33	93.33		4.98					91.93	3	2.43	2.6	70.19	4	43.52	62.0
408	Methyl ethyl ketone	SO		53.70	45.71	52.48		4.82					50.63	3	4.31	8.5	39.18	4	23.17	59.1
2582	Potassium cyanate	Other		50.12	30.90	30.20		26.92					37.07	3	11.30	30.5	34.53	4	10.53	30.5
1269	Promethazine HCl	Other		1.66	1.48	1.32		1.02					1.49	3	0.17	11.5	1.37	4	0.27	19.7
2380	Pyridine	Other		28.84	30.20	30.90		21.88					29.98	3	1.05	3.5	27.96	4	4.14	14.8
1016	Sodium hydroxide	AL		0.87	2.24	2.09		7.24					1.73	3	0.75	43.3	3.11	4	2.82	90.7
535	Sodium hydroxide	AL		14.13	26.92	8.32	21.88	4.47					17.81	4	8.23	46.2	15.14	4	9.29	61.4
2232	Sodium lauryl sulfate 3%	SU		3.02	2.88		3.24	3.09					3.05	3	0.18	5.8	3.06	4	0.15	4.8
363	Sodium lauryl sulfate 15%	SU		0.81	0.54	0.49		2.95					0.61	3	0.17	28.5	1.20	4	1.18	98.3
1721	Thiourea	Other		50.12	48.98	53.70		>5.00					50.93	3	2.47	4.8	50.93	3	2.47	4.8
1148	Trichloroacetic acid	AC		1.82	5.13	1.62		3.09					2.86	3	1.97	69.0	2.92	4	1.61	55.3
452	Trichloroacetic acid	AC		15.85	9.77	16.98		3.93					14.20	3	3.88	27.3	11.63	4	6.03	51.9
1037	Triton X-100 10%	SU		1.35	2.57	2.19		2.95					2.04	3	0.62	30.7	2.26	4	0.69	30.3
2482	Triton X-100 [1]/[2] 5%	SU		4.57	2.82	3.02		3.16					3.47	3	0.96	27.6	3.39	4	0.80	23.5
2767	Tween 20	SU		7.76	4.79	4.57		7.41					5.71	3	1.78	31.3	6.13	4	1.69	27.5
1384	[b]		not irr. at sol. (4.00)					>4.00	>4.00	>4.00	>4.00									
1769	[b]		not irr. at sol. (4.00)					>4.00	>4.00	>4.00	>4.00									
3274	[b]		not irr. at sol. (5.50)					>5.00	5.02	>5.50	>5.50	>5.50					5.02			
	Mean															**23.9**				**38.9**
	Median															**21.7**				**30.5**

[a] mean calculated using the values from assays 2-5, plus range finding assay if an actual value was obtained, i.e. < and > values were omitted.
[b] the identity of these chemicals is unknown
AC - Acid, AL - Alkaline, SO - Solvent, SU - Surfactant

3.2.2.5 COLIPA study

The COLIPA study used a set of 55 cosmetic formulations and ingredients - a large proportion of which were pure surfactants or surfactant based formulations - to assess the ability of *in vitro* methods to predict eye irritation potential. Two laboratories conducted the CM assay according to a standardized protocol (Annex A) which used an 810 second exposure time. Raw data from the studies conducted by Company # 4 and Company # 5 were obtained from the archives of Company # 3. These data are presented in Tables 3.2.2.5.a & 3.2.2.5.b (Company # 4) and Tables 3.2.2.5.c & 3.2.2.5.d (Company # 5). Raw data are found in Annexes F13 & H29.

CV's ranged from 1.3% to 55.9% (29 materials) for Company # 4 and from 1.0% to 59.4% (26 materials) for Company # 5. The mean CV for Company # 4 was 18.5% and for Company # 5 was 13.3%. The pump deodorant was the only material that seemed highly variable in both labs (CV of 48.5% [Company # 4] and 59.4% [Company # 5]). A second level of analysis was conducted to determine if there was a difference in reproducibility between surfactant and non-surfactant materials. Tables 3.2.2.5.a & 3.2.2.5.b (Company # 4) and Tables 3.2.2.5.c & 3.2.2.5.d (Company # 5) show that in both cases the variability of the surfactant materials (CV of 21.0% [Company # 4] and 14.9% [Company # 5]) was somewhat greater than the non-surfactants (CV of 13.8% [Company # 4] and 10.5% [Company # 5]).

The CV of each material for each lab was plotted against the mean MRD_{50} in Figure 3.2.2.5.a to determine if there was a relationship between the level of toxicity of a test material and its variability. The graph seems to show that the highest variability is associated with the mid-level of toxicity and that it tapers off to lower levels at the higher and lower end of the toxicity scale.

In addition, a comparison of the CV's for each material tested by both labs was made in Figure 3.2.2.5.b. If, in general, the composition of the test materials was directly related to the amount of variability found in the testing, then the within laboratory variability would be similar for each laboratory doing the testing. To test this hypothesis we graphed the CV's found in each lab against each other. It can be seen that there is a very low correlation (r^2= 0.3025) between the CV's of the 2 labs indicating that, as a general case, the composition of materials themselves did not significantly contribute to the variability. One specific exception might be the pump deodorant where between run variability was the highest (CV of 48.5% [Company # 4] and 59.4% [Company # 5]) of any of the test materials in both laboratories.

Table 3.2.2.5.a Surfactant Materials – COLIPA Intralaboratory variability of CM from archived Company # 4 data created for the COLIPA study for surfactant materials (Brantom, Bruner et al. 1997; Harbell, Osborne et al. 1999). The protocol utilized L929 cells and an 810 second exposure. N=8 for surfactant formulations. N=12 for surfactant chemicals. Data from Annex F13.

Sample ID	Substance	MRD_{50} (mg/ml)	SD	CV (%)	Number of replicates
		Surfactant Formulations			
2337	Eye make-up remover	87.77	1.17	1.3	3
3645	Gel cleaner	5.68	2.37	41.8	3
3343	Liquid soap #1	0.88	0.03	3.5	3
2429	Pump Deodorant	19.35	9.38	48.5	3
3105	Shampoo - baby	2.51	0.96	38.1	3
2092	Shampoo #1 normal	0.75	0.21	28.7	3
3213	Skin cleaner	0.63	0.1	16.3	3
Mean – Surfactant Formulations				**25.5**	
Median – Surfactant Formulations				**28.7**	
		Surfactant Chemicals			
3399	Benzalkonium chloride 1%	4.11	0.89	21.6	3
2174	Benzalkonium chloride 10%	0.32	0.07	21	3
3770	Benzalkonium chloride 5%	0.81	0.1	12.7	3
3886	Cetylpyridinium bromide 6%	1.36	0.2	14.5	3
3589	Polyethylene glycol 400	296.5	34.17	11.5	3
2721	SLS 15%	0.52	0.02	3.5	3
2089	SLS 3%	3.23	0.65	20.2	3
2079	SLS 30%	0.31	0.02	5.8	3
3740	Triton X-100 1%	21.17	4.21	19.9	3
3244	Triton X-100 10%	2.47	0.57	23	3
3806	Triton X-100 5%	4.66	0.52	11.1	3
2171	Tween 20	9.50	5.31	55.9	3
Mean – Surfactant Chemicals				**18.4**	
Median – Surfactant Chemicals				**17.2**	
Mean – All Surfactant Materials				**21.0**	
Median – All Surfactant Materials				**19.9**	

Table 3.2.2.5.b Non-Surfactant Materials – COLIPA Intralaboratory variability of CM from archived Company # 4 data created for the COLIPA study for non-surfactant materials (Brantom, Bruner et al. 1997; Harbell, Osborne et al. 1999). The protocol utilized L929 cells and an 810 second exposure. N=2 for non-surfactant formulations. N=8 for non-surfactant chemicals. Data from Annex F13.

Sample ID	Substance	MRD_{50} (mg/ml)	SD	CV (%)	Number of replicates
Non-Surfactant Formulations					
2115	Hair styling lotion	164.82	7.98	4.8	3
3525	Mouthwash	37.84	3.55	9.4	3
Mean – Non-Surfactant Formulations				**7.1**	
Median – Non-Surfactant Formulations				**7.1**	
Non-Surfactant Chemicals					
3453	Glycerol	214.83	25.35	11.8	3
2056	Imidazole	18.84	5.52	29.3	3
2356	Isopropanol	52.59	17.2	32.7	3
3870	Methyl ethyl ketone	54.18	3.16	5.8	3
3872	Propylene glycol	265.07	3.54	1.3	3
3524	Sodium hydroxide 1%	9.09	1	11	3
3631	Sodium hydroxide 10%	4.33	0.15	3.5	3
3148	Trichloroacetic acid 30%	1.12	0.31	28.1	3
Mean – All Non-Surfactant Chemicals				**15.4**	
Median – All Non-Surfactant Chemicals				**11.4**	
Mean – All Non-Surfactant Materials				**13.8**	
Median – All Non-Surfactant Materials				**10.2**	

Table 3.2.2.5.c Surfactant Materials – COLIPA Within-laboratory reproducibility of CM from archived Company # 5 data created for the COLIPA study for surfactant materials (Brantom, Bruner et al. 1997; Harbell, Osborne et al. 1999). The protocol utilized L929 cells and an 810 second exposure. N=8 for surfactant formulations. N=10 for surfactant chemicals. Data from Annex H29.

Sample ID	Substance	MRD_{50} (mg/ml)	SD	CV (%)	Number of replicates
	Surfactant Formulations				
2201	Eye make-up remover	99.31	1	1	3
3677	Gel cleaner	5.47	1.2	22	3
2254	Hair styling lotion	292.01	6.07	2.1	3
2003	Pump Deodorant	47.74	28.34	59.4	3
3306	Shampoo – baby	2.15	0.73	33.7	3
3440	Shampoo #1 normal	0.72	0.06	8.1	3
3328	Skin cleaner	0.76	0.05	6	3
2386	Liquid Soap #1	0.68	0.10	14.0	3
Mean – Surfactant Formulations				**18.3**	
Median – Surfactant Formulations				**11.1**	
	Surfactant Chemicals				
3517	Benzalkonium chloride 1%	4.33	1.19	27.4	3
2901	Benzalkonium chloride 10%	0.31	0.05	16.4	3
2811	Benzalkonium chloride 5%	1.38	0.12	8.9	3
3825	Polyethylene glycol 400	>316.23	ND	ND	2
3191	SLS 15%	0.51	0.02	3.3	3
2136	SLS 3%	2.78	0.07	2.7	3
3207	Triton X-100 1%	16.79	0.73	4.3	3
3720	Triton X-100 10%	1.24	0.28	22.9	3
3500	Triton X-100 5%	2.42	0.07	2.7	3
3561	Tween 20	3.49	0.62	17.7	3
Mean – Surfactant Chemicals				**10.6**	
Median – Surfactant Chemicals				**6.6**	
Mean – All Surfactant Materials				**14.9**	
Median – All Surfactant Materials				**8.9**	

Table 3.2.2.5.d Non-Surfactant Materials – COLIPA Within-laboratory reproducibility of CM from archived Company # 5 data created for the COLIPA study for surfactant materials (Brantom, Bruner et al. 1997; Harbell, Osborne et al. 1999). The protocol utilized L929 cells and an 810 second exposure. N=2 for non-surfactant formulations. N=7 for non-surfactant chemicals. Data from Annex H29.

Sample ID	Substance	MRD_{50} (mg/ml)	SD	CV (%)	Number of replicates
		Non-Surfactant Formulations			
2254	Hair styling lotion	292.01	6.07	2.1	3
2563	Mouthwash	46.85	9.2	19.6	3
Mean – Non-Surfactant Formulations				10.9	
Median – Non-Surfactant Formulations				**10.9**	
		Non-Surfactant Chemicals			
2479	Glycerol	208.7	3.06	1.5	3
3337	Imidazole	26.03	0.99	3.8	3
3789	Isopropanol	124.51	25.26	20.3	3
3556	Propylene glycol	218.86	7.59	3.5	3
3357	Sodium hydroxide 1%	13.59	5.11	37.6	3
3434	Sodium hydroxide 10%	0.6	0.01	1.9	3
3864	Trichloroacetic acid 30%	1.24	0.05	4.2	3
Mean – Non-Surfactant Chemicals				10.4	
Median – Non-Surfactant Chemicals				**3.8**	
Mean – All Non-Surfactant Materials				10.5	
Median – All Non-Surfactant Materials				**3.8**	

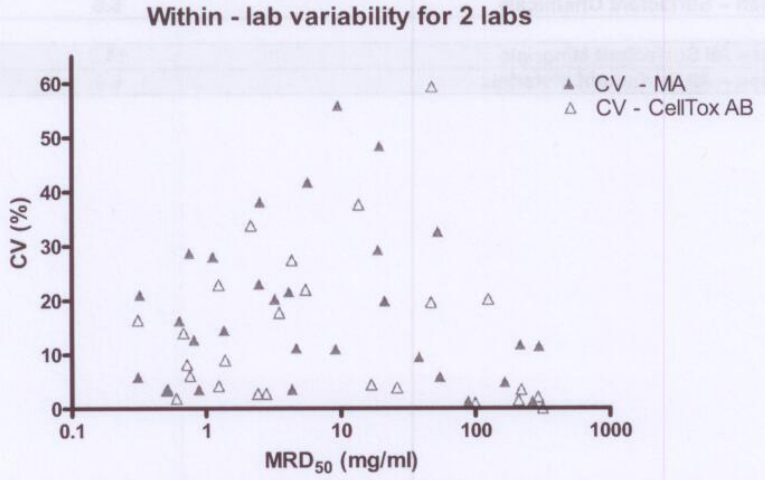


Figure 3.2.2.5.a **Graph of the variability of CM data for the two laboratories participating in the COLIPA study. The CV for each test material measurement is plotted against the mean MRD_{50} value for that material. Twenty-nine materials are plotted for Company # 4 and 26 materials for Company # 5.**

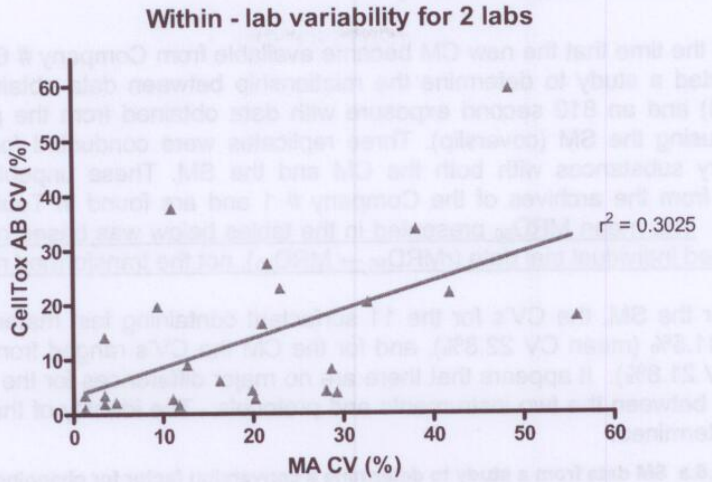


Figure 3.2.2.5.b Graph of the coefficient of variation of CM data for the two laboratories participating in the COLIPA study. The CV obtained for each test material at Company # 5 is plotted against the CV obtained for each test material at Company # 4

3.2.2.6 SM and CM comparison study

At the time that the new CM became available from Company # 6, the Company # 1 conducted a study to determine the relationship between data obtained with the CM (transwell) and an 810 second exposure with data obtained from the prior 500 second protocol using the SM (coverslip). Three replicates were conducted for each of eleven proprietary substances with both the CM and the SM. These unpublished data were obtained from the archives of the Company # 1 and are found in Tables 3.2.2.6.a and 3.2.2.6.b. The mean MRD_{50} presented in the tables below was based on the average of transformed individual trial data ($rMRD_{50} \rightarrow MRD_{50}$), not the transformed mean $rMRD_{50}$.

For the SM, the CV's for the 11 surfactant containing test materials ranged from 1.7% to 41.5% (mean CV 22.8%), and for the CM the CV's ranged from 3.7% to 47.7% (mean CV 21.8%). It appears that there are no major differences for the within-laboratory variability between the two instruments and protocols. The identity of the materials could not be determined.

Table 3.2.2.6.a SM data from a study to determine a conversion factor for changing SM (500 sec exposure) values to CM (810 sec exposure) values. N = 11 materials. Data from Annex F35.

Substance	MRD_{50} (mg/mL)	SD	CV (%)	Number of replicates
A	21.3	3.79	17.7	3
B	0.083	0.00	1.7	3
C	0.273	0.02	5.5	3
D	0.283	0.12	41.5	4
E	12.0	2.27	18.9	3
F	0.032	0.01	28.2	3
G	0.143	0.05	36.8	3
H	1.62	0.39	24.2	3
I	0.038	0.01	14.7	3
J	0.036	0.01	26.9	3
K	0.109	0.04	34.5	3
Mean			**22.8**	
Median			**24.2**	

Table 3.2.2.6.b CM data from a study to determine a conversion factor for changing SM (500 sec exposure) values to CM (810 sec exposure) values. N = 11 materials. Data from Annex F35.

Substance	MRD_{50} (mg/mL)	SD	CV (%)	Number of replicates
A	55.8	7.00	12.5	3
B	0.320	0.09	28.4	3
C	0.722	0.15	20.4	3
D	0.428	0.02	3.7	3
E	8.61	3.08	35.8	3
F	0.067	0.01	19.2	3
G	0.244	0.06	24.3	3
H	2.19	0.23	10.6	3
I	3.21	1.53	47.7	3
J	0.158	0.01	10.6	3
K	0.236	0.07	28.4	3
Mean			**21.8**	
Median			**20.4**	

3.2.2.7 Materials common to the EC/HO and the COLIPA study (Type II reproducibility)

Sixteen of the twenty materials which were common to both the EC/HO study and the COLIPA study provide the opportunity to investigate longer-term within lab reproducibility (or type II reproducibility) for the laboratory of Company # 4 Nearly two years elapsed between the initiation of the EC/HO study (April 1993) and the initiation of the COLIPA study (January 1995) at Company # 4 Although the materials had similar identities, it should be noted that they were purchased at different times (approximately 2 years apart) and could have had different purities or compositions (for the materials that are only broadly described mixtures of different sized polymers). Tables 3.2.2.7.a and 3.2.2.7.b show the results for 11 surfactant materials and 9 non-surfactants, respectively. Two materials were determined to be compatible with testing with the microphysiometer instrument in use for the EC/HO study but not for the COLIPA study, while one material was determined to be testable in the COLIPA study, but not the EC/HO study. One of the common materials was judged not testable for both studies. Thus there was fairly good correlation on the testing decisions for both studies even though the criteria for "testability" was more carefully defined in the COLIPA protocol than it was in the EC/HO protocol.

For the 9 surfactants and 7 non-surfactants that were actually tested in both studies, the CV's ranged from 0.6% to 37.7% (mean CV 17.4%) for surfactants and 5.0% to 65.1% (mean CV 32.5%) for the non-surfactants. The reproducibility for the surfactants was very similar for the Type I and Type II situations (mean CV of 19.5% (Company # 4); COLIPA study] and 26.11% [SM31 (Company # 4); EC/HO study], respectively). The higher variability for the non-surfactant materials was primarily due to the variability of the three highly acidic or basic materials – 1% sodium hydroxide, 10% sodium hydroxide, and 30% trichloroacetic acid.

Tables 3.2.2.7.a & 3.2.2.7.b show MRD_{50} results for materials of similar identity. It appears from these data that long term within lab reproducibility is good for the surfactant materials; however, this conclusion is based on CVs determined from only two measurements per test material (mean COLIPA value vs. mean EC/HO value) and therefore should not be over-interpreted. The mean MRD_{50} presented in the tables below was based on the transformed mean log MRD_{50} given in the Bibra Preliminary Report on the EC/HO study, not the average of transformed individual trial data ($rMRD_{50} \rightarrow MRD_{50}$).

Table 3.2.2.7.a Surfactant materials - Comparison of the MRD_{50} values for testing conducted approximately 21 months apart. N = 11 surfactant materials. Data from Annex H7 & F13.

Substance	COLIPA Mean MRD_{50} (mg/mL) [CV%] MA	EC/HO Mean MRD_{50} (mg/mL) SM 31	Mean MRD_{50} (mg/mL)	SD	CV (%)
Surfactant Chemicals					
Benzalkonium chloride 1%	4.11	5.16	4.62	0.72	15.6
Benzalkonium chloride 10%	0.321	0.47	0.39	0.1	26.3
Benzalkonium chloride 5%	0.811	1.09	0.96	0.2	21.4
Cetylpyridinium bromide 10%	*	1.02	1.02	*	*
Cetylpyridinium bromide 6%	1.36	1.35	1.35	0.01	0.6
Polyethylene glycol 400	296.5	*	296.5	*	*
Sodium lauryl sulphate 15%	0.517	0.60	0.56	0.06	10.9
Sodium lauryl sulphate 3%	3.23	3.04	3.13	0.15	4.8
Triton X-100 10%	2.47	1.96	2.21	0.37	16.6
Triton X-100 5%	4.66	3.39	4.03	0.9	22.3
Tween 20	9.50	5.53	7.5	2.83	37.7
Mean – All Surfactant Chemicals					**17.4**
Median – All Surfactant Chemicals					**16.6**

* - Material determined to be unsuitable for testing

Table 3.2.2.7.b Non-surfactant materials - Comparison of the MRD_{50} values for testing conducted approximately 21 months apart. N = 9 non-surfactant chemicals. Data from Annex H7 & F13.

Substance	COLIPA Mean MRD_{50} (mg/mL) [CV%] MA	EC/HO Mean MRD_{50} (mg/mL) SM 31	Mean MRD_{50} (mg/mL)	SD	CV (%)
Non-Surfactant Chemicals					
Ethyl acetate	*	53.7	53.7	*	*
Glycerol	214.8	180.7	197.75	24.11	12.2
Imidazole	18.8	23.1	20.95	3.04	14.5
Isopropanol	52.6	91.2	71.9	27.29	38
Methyl ethyl ketone	54.2	50.5	52.35	2.62	5
n-Butyl acetate	*	*	*	*	*
Sodium hydroxide 1%	9.09	16.2	12.65	5.03	39.8
Sodium hydroxide 10%	4.33	1.60	2.97	1.93	65.1
Trichloroacetic acid 30%	1.12	2.47	1.8	0.95	53.2
Mean – All Non-Surfactant Chemicals					**32.5**
Median – All Non-Surfactant Chemicals					**38.0**

* - Material determined to be unsuitable for testing

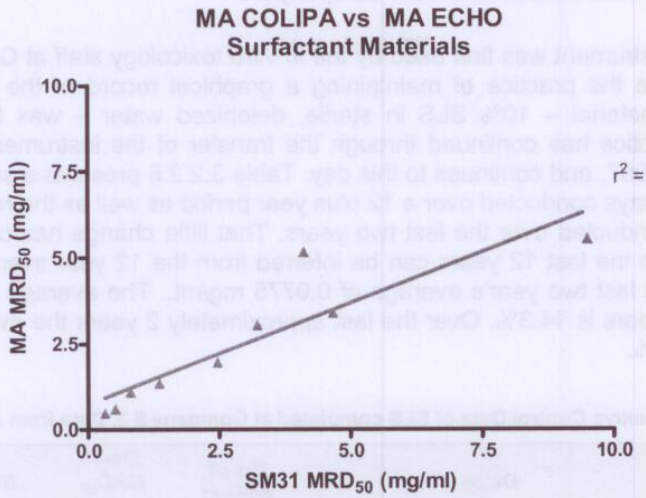


Figure 3.2.2.7.a Surfactant materials for comparison between inter-laboratory data of COLIPA and EC/HO studies.

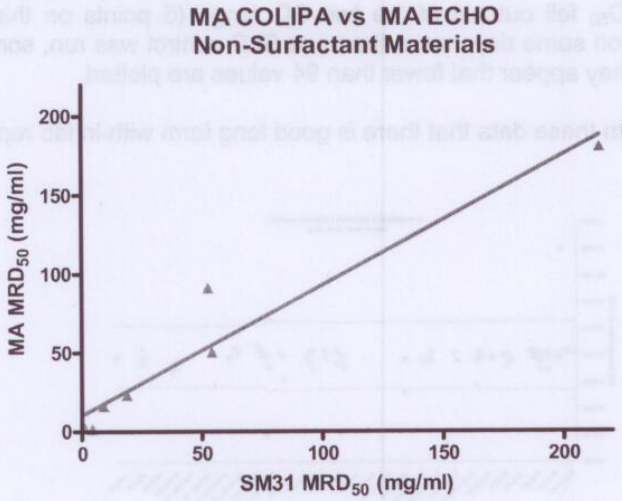


Figure 3.2.2.7.b Non-surfactant materials for comparison between inter-laboratory data of COLIPA and EC/HO studies.

3.2.2.8 Historic positive control data from Company # 3

The CM instrument was first used by the *in vitro* toxicology staff at Company # 4 in 1994. At that time the practice of maintaining a graphical record of the results of the positive control material – 10% SLS in sterile, deionized water – was begun (Figure 3.2.2.8). This practice has continued through the transfer of the instrument and staff to Company # 3 in 1997, and continues to this day. Table 3.2.2.8 presents a summary of the results for 629 assays conducted over a 12 plus year period as well as the results from the last 94 assays conducted over the last two years. That little change has occurred in the absolute MRD_{50} in the last 12 years can be inferred from the 12 year average of 0.0799 mg/mL versus the last two year's average of 0.0775 mg/mL. The average CV calculated over the last 12 years is 14.3%. Over the last approximately 2 years the average CV has increased to 18.9%.

Table 3.2.2.8 Positive Control Data of SLS completed at Company # 3. Data from Annex H17.

Substance	Dates	No. of Assays	Mean MRD_{50} (mg/mL)	SD	CV (%)
SLS	April, 14 1994 – June 30, 2006	629	0.0799	0.011	14.3
SLS	March 2, 2004 - June 30, 2006	94	0.0775	0.015	18.9

SLS MRD_{50} values are plotted on a control graph with upper and lower cut-off ranges graphed at two SD of all data (March 2004 – June 2006). Assays performed on days when the MRD_{50} fell outside of the two SD range (5 points on this graph) were repeated. Because on some days more than one SLS control was run, some points may overlap such that it may appear that fewer than 94 values are plotted.

It appears from these data that there is good long term with-in lab reproducibility for a single material.

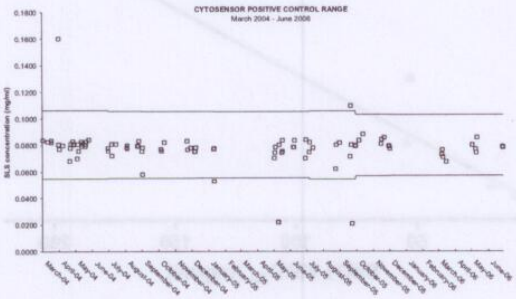


Figure 3.2.2.8 Graph of 10% SLS (positive control) MRD_{50} values obtained at Company # 3 over a 28-month period.

3.2.3 Additional information/evaluation – Operator Variability

Operator variability was assessed using the positive control data collected from technicians performing the Cytosensor Microphysiometer assay at Company # 3. The average MRD_{50} (mg/mL), standard deviation, and coefficient of variance is presented in Table 3.2.3 for each of the eight technicians. Raw data are in Annex H25. The mean MRD_{50} collected for the eight technicians was 0.0795 mg/mL, with a mean coefficient of variation of 12.6%. Although different batches of SLS were used between the operators, the CV remains low which indicates a low degree of operator variability for the Cytosensor Microphysiometer when using the same protocol.

Table 3.2.3 Operator variability assessed using the positive control data at Company # 3. N = 1 material. See Annex H25.

Operator Number	Number of Experiments	Average MRD_{50} (mg/mL)	Standard Deviation	CV (%)
1	49	0.0830	0.0050	6.03
2	110	0.0792	0.0082	10.31
3	32	0.0760	0.0047	6.14
4	76	0.0814	0.0228	28.06
5	44	0.0791	0.0073	9.18
6	80	0.0781	0.0102	13.07
7	18	0.0779	0.0166	21.25
8	7	0.0773	0.0055	7.15
Total for all operators	416	0.0795	0.0125	12.6

3.3 Additional studies where raw data are not available: attempt to combine the data using weigh-of-evidence approaches

There were no other studies which we could find which would allow any inferences about within laboratory reproducibility. The one other important SM study (Bagley, Bruner et al. 1992) described elsewhere in this BRD reports only average MRD_{50}'s and provides no information on either number of trials or the reproducibility of those trials.

3.3.1 Protocols and long term intralaboratory reproducibility

Sixteen of the twenty materials which were common to both the EC/HO study and the COLIPA study provide the opportunity to investigate longer-term within lab reproducibility (or type II reproducibility) for Company # 4 laboratory. Nearly two years elapsed between the initiation of the EC/HO study (April 1993) and the initiation of the COLIPA study (January 1995) at Company # 4 Thus there was fairly good correlation on the testing decisions for both studies even though the criteria for "testability" was more carefully defined in the COLIPA protocol than it was in the EC/HO protocol. The

correlation coefficient between surfactants tested in both the COLIPA and EC/HO studies was 0.8141. The correlation coefficient between non-surfactants tested in both the COLIPA and EC/HO studies was 0.9322.

There were no major discrepancies for the within-laboratory variability between the SM and CM and their respective protocols (Table 3.2.2.6.a and 3.2.2.6.b). For the SM, the CV's for the 11 test materials ranged from 1.7% to 41.5% (mean CV 22.8%), and for the CM the CV's ranged from 3.7% to 47.7% (mean CV 21.8%).

Company # 4 was included in three different studies, the CTFA, EC/HO, and COLIPA. Although all three studies had different protocols, the CV was approximately the same. Therefore, the difference in protocol did not have a significant effect on the MRD_{50} values (mean CV 27.5%).

3.3.2 Test materials

For the similar chemicals tested in the COLIPA and EC/HO study at Company # 4 21 months apart, the non-surfactant chemicals had a slightly higher CV (32.5%) than the surfactant and surfactant containing test materials (17.4%). However, during the COLIPA study, the surfactants had a slightly higher CV for both laboratories (19.5% and 26.11%) than the non-surfactants (17.5% and 28.9%). The study from Company # 2 tested only surfactants for a CV of 23.0%. The surfactants and surfactant containing materials had a consistent CV of approximately 20% between three different protocols (Company # 2, COLIPA, and EC/HO); however, the non-surfactants had a varying CV ranging from 65.1% to 5.0% between the two different protocols (COLIPA and EC/HO).

When looking at the MRD_{50} of the COLIPA study versus the MRD_{50} of the EC/HO study (Figure 3.2.2.7.a and b), the correlation coefficient for the surfactants was slightly lower at 0.8141 than for the non-surfactants 0.9322. However, the number of materials for each set was small (9 and 7 for the surfactants and non-surfactants, respectively).

3.3.3 Classifications

To determine if reproducibility was related to the degree of irritation (by MRD_{50} rank), we plotted the percent CV against the MRD_{50} for the COLIPA, EC/HO, CTFA, and Bagley studies in Figure 3.3.3.a. There appeared to be no strong correlation between MRD_{50} and CV, however there were a few more of the extremely high CV values (>70) for materials in the moderate to non-irritating (MRD_{50} = 1 – 300 mg/ml) range.

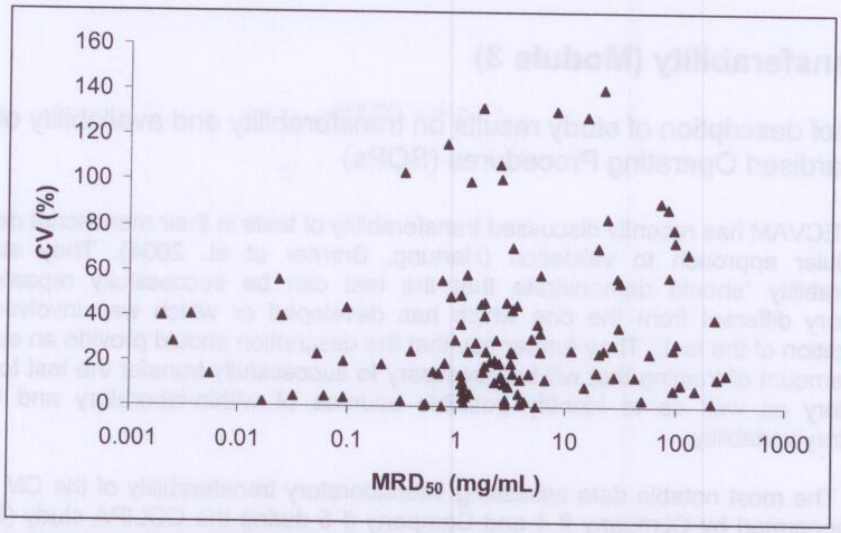


Figure 3.3.3.a Comparison of CV and MRD_{50} for the COLIPA, EC/HO, CTFA and the Bagley studies.

4. Transferability (Module 3)

4.1 Brief description of study results on transferability and availability of Standardised Operating Procedures (SOPs)

ECVAM has recently discussed transferability of tests in their manuscript describing a modular approach to validation (Hartung, Bremer et al. 2004). They state that transferability "should demonstrate that the test can be successfully repeated in a laboratory different from the one which has developed or which was involved in the optimization of the test." They further say that the description should provide an estimation of the amount of training that will be necessary to successfully transfer the test to a naïve laboratory as well as to identify possible sources of within-laboratory and between laboratory variability.

The most notable data assessing interlaboratory transferability of the CM protocol were generated by Company # 4 and Company # 5 during the COLIPA study (Brantom, Bruner et al. 1997). The data from this study indicate that a thorough and precise protocol can be transferred between laboratories with success. Before the study began, both laboratories were given a common protocol which addressed decision criteria for which materials could be tested, as well as detailed procedures for actual testing. The protocol was discussed in detail between the study directors for each laboratory, sources of potential variability were identified, and methods to approach potential problems were addressed. However, we were unable to find any written records of these discussions. No formal laboratory training sessions were conducted. The protocol for these studies can be found in Annex A.

During the conduct of the actual COLIPA study, communications between the two laboratories were kept to a minimum to ensure that the results of the study clearly reflected the reproducibility that could be expected from two completely independent laboratories conducting any set of studies. Test materials were coded in a double blind fashion, i.e. both laboratories had different code designations for identical test materials, by a third party so that results for a test material could not be easily be discussed between laboratories.

As can be seen in Table 4.1.a, between-laboratory reproducibility (the CV for the mean MRD_{50}s for each laboratory) ranged from 1.0% - 106.9% with a mean CV of 24.7%. It should be noted that the highest CV (106.9%) was found for a high concentration of base (10% NaOH) which might be expected in a dilution-based assay conducted in a medium with minimal buffering capacity. However, 1% NaOH and 30% trichloroacetic acid were much more reproducible. Section 3 (Within-laboratory reproducibility) of this BRD also addressed reproducibility difficulties for materials that were strongly acidic or basic.

The mean between-laboratory CV for these studies (24.7%; 21.4% if the results for 10% NaOH are removed) was somewhat higher than the individual within-laboratory CV (18.5% for MA, 13.1% for CT) for the chemicals and formulations that were tested by both

laboratories. These data would seem to indicate that a second laboratory (Company # 5) can successfully repeat data from a laboratory that was involved in the optimization of the test (Company # 4).

Table 4.1.a Results from Company # 4 and Company # 5 laboratories for the COLIPA study demonstrating the transferability of the Cytosensor Microphysiometer protocol. N = 10 surfactant chemicals, N = 7 surfactant formulations, N = 7 Non-surfactant chemicals, and N = 2 non-surfactant formulations. Data from Annex F13 & H29.

Chemical	Conc. tested	MRD_{50} Values (mg/mL)		Number of Replicates		Mean MRD_{50}	SD MRD_{50}	CV (%)
		Company # 4	Company # 5	Company # 4	Company # 5			
Surfactant Chemicals								
Benzalkonium chloride 1%	1%	4.11	4.33	3	3	4.22	0.16	3.7
Benzalkonium chloride 10%	10%	0.32	0.31	3	3	0.31	0.01	3.2
Benzalkonium chloride 5%	5%	0.81	1.38	3	3	1.1	0.4	36.7
Polyethylene glycol 400	100%	296.5	316.23	3	3	306.36	13.95	4.6
SLS 15%	15%	0.52	0.51	3	3	0.51	0.01	1
SLS 3%	3%	3.23	2.78	3	3	3	0.32	10.6
Triton X-100 1%	1%	21.17	16.79	3	3	18.98	3.1	16.3
Triton X-100 10%	10%	2.47	1.24	3	3	1.85	0.87	46.8
Triton X-100 5%	5%	4.66	2.42	3	3	3.54	1.58	44.7
Tween 20	100%	9.5	3.49	3	3	6.5	4.25	65.4
Mean – Surfactant Chemicals								**23.3**
Median – Surfactant Chemicals								**13.5**
Surfactant Formulations								
Eye make-up remover	100%	87.77	99.31	3	3	93.54	8.16	8.7
Gel cleaner	100%	5.68	5.47	3	3	5.58	0.15	2.6
Liquid soap #1	100%	0.88	0.68	3	3	0.78	0.14	18.5
Pump Deodorant	5%	19.35	47.74	3	3	33.54	20.08	59.9
Shampoo – baby	100%	2.51	2.15	3	3	2.33	0.25	10.8
Shampoo #1 normal	100%	0.75	0.72	3	3	0.74	0.02	2.2
Skin cleaner	100%	0.63	0.76	3	3	0.7	0.09	13
Mean – Surfactant Formulations								**16.5**
Median – Surfactant Formulations								**10.8**
Non-Surfactant Chemicals								
Glycerol	100%	214.83	208.7	3	2	211.77	4.34	2
Imidazole	100%	18.84	26.03	3	3	22.43	5.09	22.7
Isopropanol	100%	52.59	124.51	3	3	88.55	50.86	57.4
Propylene glycol	100%	265.07	218.86	3	3	241.97	32.67	13.5
Sodium hydroxide 1%	1%	9.09	13.59	3	3	11.34	3.19	28.1
Sodium hydroxide 10%	10%	4.33	0.6	3	3	2.47	2.64	106.9
Trichloroacetic acid 30%	30%	1.12	1.24	3	3	1.18	0.09	7.3
Mean – Non-Surfactant Chemicals								**34.0**
Median – Non-Surfactant Chemicals								**22.7**
Non-Surfactant Formulations								
Hair styling lotion	100%	164.82	292.01	3	3	228.41	89.94	39.4
Mouthwash	100%	37.84	46.85	3	3	42.35	6.37	15
Mean – Non-Surfactant Formulations								**27.2**
Median – Non-Surfactant Formulations								**27.2**
Mean – All Materials								**24.7**
Median – All Materials								**14.3**

Two Standard Operating Procedures (SOPs) are available for this method from ECVAM SIS, INVITOX #102 "The Silicon Microphysiometer Toxicity Test - Procter and Gamble" written by Dr. Rosemarie Osborne and INVITOX #97 "The Silicon Microphysiometer Toxicity Test - Microbiological Associates" written by Dr. John Harbell. There is also a protocol outline available for the Institute For In Vitro Sciences Cytosensor Microphysiometer Bioassay; however, this document does not describe the test method in as much detail as the SOPs. The INVITOX SOPs describe the method in detail, but were submitted in 1996 so a number of modifications are necessary to both documents. A list of the major revisions that we suggest are necessary to be made to the Company # 4 SOP are listed in the table below (Table 4.1.b). The same revisions are applicable to the Procter and Gamble SOP also.

Table 4.1.b Major revisions to the Company # 4 SOP

SOP Section	Modification
Test Status – first six sentences	"The Cytosensor Microphysiometer, manufactured by Company # 6, measures alterations in the acidification rate of cells. The microphysiometer consists of a variety of components which include (1) Cytosensor Microphysiometer Unit(s), (2) "Cytosoft" – the computer program which runs the microphysiometer and collects the data, (3) sensor chambers, and (4) a printer. Various adherent cell types can be grown on a capsule cup, which is a disposable cup with a polycarbonate membrane with a 3 µm pore size. A spacer (circular disk) is added to the capsule cup and this assembly (cell capsule) is added to the sensor chamber. The silicon chip within the sensor chamber is capable of detecting very small changes in pH."
Procedure Details – Starvation Medium	Change to Seeding medium which contains 1% Fetal Bovine Serum
Procedure Details – Route of Administration	The test article is exposed for 810 seconds, followed by an approximate 300 second rinse, and an approximate 20 second rate measure
Procedure Details – Growth of Cells	The L929 cells are seeded in capsule cups using the seeding medium the day before the dosing is to be initiated. The cell capsules and spacer are placed into the microphysiometer flow chambers and exposed to MDMEM at 37 ±1°C prior to dosing.
Procedure Details – Dose selection – second and third paragraphs	The exposure time for each test material dilution is 810 seconds. The test article doses for the definitive assay are chosen so that at least seven treatments are available for the determination of the MRD_{50}.

4.2 Facilities and major fixed equipment needed

In order to perform the Cytosensor Microphysiometer assay, equipment for both cell culture (Table 4.2.a) and specialized assay equipment (Table 4.2.b) are necessary. Company # 6 no longer supports the Cytosensor (i.e. provides repair services and replacement parts) as of June 2007. Sale of replacement parts will continue after this time only until the supply of parts is exhausted. Company # 6 will not procure any more parts or disposables after this date. They have also informed us that the names of their current suppliers for replacement parts will not be revealed to any current user.

These changes will obviously have a severe impact on anyone who is just now anticipating taking up the assay. Company # 3 laboratory has sufficient spare parts and disposables to last for several years, but after this time it is likely that the machine cannot be supported.

Table 4.2.a Equipment for cell culture

Equipment	Use
Laminar flow hood	Cell culture manipulations
Incubator, 37°, 5% CO_2, 90% humidity	Cell culture incubation
Cell Counter or Hemacytometer	Performing cell counts
Inverted Microscope	Observing the confluence of cell cultures
Water bath	Warming cell culture materials
Aspirator	Removal of media during routine cell culture and passaging
Refrigerator	Storing chemicals and reagents
Freezer, liquid nitrogen container	Storage of medium components and cell banks
Pipettes	Adding test materials and cell culture media

Table 4.2.b Specialized assay equipment

Equipment	Use
Company # 6 Cytosensor Unit	Automated dosing, rinsing, and data collection for the assay
Sensor Chamber	The capsule kit is added to this part of the Cytosensor Unit which includes the pH sensor device that collects data for the assay
Cytosoft computer program (see the paragraph below for additional information)	Software used to run the Cytosensor Unit and collect and calculate data
Debubbler membranes	Warmed to 6°C above the temperature of the sensor chamber to allow dissolved gases to leave the fluid and escape through the membrane

Reference Electrode Maintain Kit (KCl)	Stabilizes pH readings from the sensor chamber
Spacers	Defines the height and diameter of the compartment in which the cells are confined
Sterilant Solution Kit	To sterilize the Cytosensor Unit after experimentation is completed
Capsule Cups, 12 mm, 3 µm pore size	Immobilize living cells in the sensor chamber while allowing fluid movement through the cell layer
Balance	Weighing test materials for accurate dosing solution preparation

The Cytosoft program runs on a Macintosh IIsi. At least 5 megabytes of RAM (a minimum of which 2.5 megabytes must be allocated for the Cytosoft program) are needed along with a floating-point processor and 32-bit QuickDraw System 7.0 or higher to run this program. Two and a half megabytes of RAM will allow Cytosoft to gather and save data for 13 hours. It is recommended that all other software be closed and that Cytosoft is the only program running on the computer while data are being collected. If other programs are running, there is a risk of causing printing errors and/or software crashes.

The newest version of the software is Cytosoft 2.03 which can run on Mac OS 9, but has not been tested with OS X (Personal communication; Company # 6). Company # 6 states that both the Mac G3 and G4 computers can be used to operate the CM.

4.3 Required level of training, expertise, and demonstrated proficiency needed

The following discussion is based primarily on the 12-plus years of experience that staff at Company # 3 have had with the machine, as well as past conversation with other users.

Basic training in sterile cell culture technique is essential for this assay. Individuals wishing to perform this assay must demonstrate proficiency in the propagation of the L929 cells used in this assay. These procedures include training on utilization of a hemacytometer or Coulter Counter for calculation of the proper seeding density. A possible source of within and between laboratory variability is controlling the cell seeding so that the protocol requirement of a 50 to 200 µvolt/sec acidification rate can be routinely achieved. Also, individuals wishing to perform the Cytosensor Microphysiometer assay must be familiar with the preparation of cell culture media and must follow the proper protocol or SOP and construct a batch record to record the components of and steps taken to manufacture this media.

In addition to this basic sterile technique and cell culture training, individuals should demonstrate proficiency in performing serial dilutions and must be trained in the use of the Cytosoft software. Special attention should be paid to proper training in the test article dilution techniques to ensure that accurate dilutions are prepared, as this is another area

of possible variation within and between laboratories. In the experience of Company # 3, the training is usually comprised of watching an experienced technician set up the assay, create dilutions of the test articles and controls, and run the proper protocol once or twice followed by hands-on performance of the techniques for 2 to 3 assays under the direction of an experienced technician. Where appropriate, additional training in the analysis of the data via an Excel spreadsheet to determine the MRD_{50} value is also necessary. A working knowledge of Good Laboratory practices (GLPs) may also be helpful. Proper training and competency in the performance of this assay can be demonstrated by successfully running a positive control (e.g. SLS) and obtaining results within a predetermined historical mean or within the margin of error for the data generated during the COLIPA study mentioned previously.

The table below (Table 4.3) represents data obtained during training for various technicians performing the Cytosensor Microphysiometer assay at Company # 3. The SOPs and protocol remained essentially the same over time and between technicians. This data demonstrates the ability to teach the method to naïve individuals and obtain similar results after successful completion of a monitored training period. The MRD_{50} value obtained during these technician training runs is presented along with the batch of SLS used and the date on which the training was performed.

Table 4.3 Example of the MRD_{50} values for SLS obtained during the training of technicians at Company # 3. Data from internal records at Company # 3.

Date	Technician	Batch #	MRD_{50} (mg/mL)
02/26/99	Technician 1		0.0754
02/26/99	Technician 1		0.0967
04/23/03	Technician 2	40	0.0807
04/23/03	Technician 2	43	0.0953
04/24/03	Technician 2	40	0.0811
04/24/03	Technician 2	40	0.0831
04/24/03	Technician 2	43	0.0789
04/24/03	Technician 2	43	0.0846
08/28/03	Technician 3	43	0.0806
08/29/03	Technician 3	43	0.0799
08/24/05	Technician 4	59	0.0775
08/24/05	Technician 4	59	0.0782
08/24/05	Technician 4	61	0.0915
08/24/05	Technician 4	61	0.0780
08/25/05	Technician 4	61	0.0778
08/25/05	Technician 4	61	0.0622
03/02/06	Technician 4	62	0.0955
03/02/06	Technician 4	62	0.0870
03/02/06	Technician 5	62	0.0879
03/16/06	Technician 4	63	0.0711
03/16/06	Technician 5	63	0.0724
		Average	**0.0817**
		SD	0.0086
		CV (%)	10.5

5. Between-laboratory reproducibility (Module 4)

The SM and CM assays have been used by more than one laboratory in two major international studies and one more restricted study (see Table 5.1) which allowed between-laboratory reproducibility to be assessed. The EC/HO study had four laboratories (Department of Chemistry, Stanford University [Palo Alto, CA, USA]; Company # 2; Company # 4 and Company # 1) using the CM with the 810 second transwell protocol. The COLIPA study had two laboratories (Company # 5 and Company # 4) using the CM which also had an 810 second protocol. The restricted study of Bagley, Bruner et al. 1992 would not normally technically qualify for analysis in this section since the machines used by the participating laboratories (Company # 4 and Company # 2) were different. One laboratory used the SM fitted with a transwell cell chamber, and one used the SM with the glass coverslips. However, the other important factors in the protocols, e.g treatment time, cell line, general operation, were virtually identical making it useful to evaluate the reproducibility of the general method within this BRD.

5.1 Relevant results and information for studies where raw data is available

Table 5.1 Table presenting the relevant results and information for studies where raw data are available

Studies	Number of chemicals tested	Number of products tested	Instrument	Coded ?	Results reported (Range)	Replicates		Data format	Chemical classes tested	Ranges of toxicity covered	Physico-chemical properties covered
						n. of labs	n. of reps				
Several companies[1] (Bagley, Bruner et al. 1992)	12 of 12 tested	20 of 20 tested	SM (2 Protocols)	Yes	MRD_{50} (mg/mL) 0.002-15.364	2	3	Summary; MAS and log MRD_{50} only	Cleaning product ingredients-almost all surfactants	MAS 0.3 – 43 for analysis; 0 – 44.7 total	See Annex B, C, & D
EC/HO[2] (Balls, Botham et al. 1995)	31 – 38 (depending on lab) of 60 were compatible	0	CM	Yes	MRD_{50} (mg/mL) 0.39-363.08	4	3	Individual log MRD_{50} data; raw animal data	Alcohols, surfactants, ketones, bases, acids, esters	MMAS 0 – 108 EU: R36, R41, NC GHS : 1, 2A, 2B, NC EPA: I-IV	See Annex B, C, & D
COLIPA[3] (Brantom, Bruner et al. 1997)	17-20 (depending on lab) of 23 were compatible	9 of 32 were compatible	CM	Yes	MRD_{50} (mg/mL) 0.31-306.36	2	3	Individual log MRD_{50} data; raw animal data	Alcohols, surfactants, ketones, bases, acids, esters	MAS 0 – 106 EU: R36, R41, NC GHS : 1, 2A, 2B, NC EPA: I-IV	See Annex B, C, & D

[1] Performed using the silicon microphysiometer coverslip protocol and silicon microphysiometer transwell protocol with a 500-second exposures and L929 cells.
[2] Performed using the Cytosensor transwell protocol with an 810-second exposure and L929 cells.
[3] Performed using the Cytosensor transwell protocol with an 810-second exposure and L929 cells.

5.2 Discussions, e.g., were sources of variability taken into consideration

There was not sufficient information available from the three studies to investigate such items as time frame of the assay, different media suppliers, and level of adherence to the protocol. However, for both the COLIPA and EC/HO studies a detailed protocol was available and all participating laboratories agreed to conduct the study according to this document. Variables which are known are discussed in the individual study sections below.

5.2.1 Bagley, Bruner et al. 1992 study

Although the main purpose of this study was to determine the utility of the SM assay to predict the ocular irritation potential of 32 coded test materials, a secondary goal was to compare data from the traditional SM device with a prototype machine similar to what would later be the CM. Most of the protocol parameters were conserved between the two laboratories, e.g. L929 cells and a 500 second exposure period were used in both. However, the cells were plated on glass cover slips for exposure in one laboratory while the cells were plated on the permeable membrane of a transwell in the other laboratory. Although the medium and serum were purchased from the same company – GIBCO – one batch was produced in the US and the other in France.

A correlation analysis of the results from both laboratories was conducted by the study authors, and a very good correlation (Pearson/Spearman correlation coefficients 0.93/0.90 and all points lying close to a line of unity) were reported. As expected from their close correlation with each other, both assays also gave similar relatively high correlation coefficients with the Draize scores of the 32 test materials (Pearson/Spearman of 0.82/0.75 for the SM and 0.86/0.81 for the CM).

Table 5.2.1.a and 5.2.1.b display the mean, SD, and CV for results from each report for each of the 32 test materials. Fifty-six percent of the interlaboratory CV's were <25%. The results of this study indicate that when testing chemical ingredients, household cleaning products, personal care products, and cosmetics, the protocol used was very reproducible even when two different machine configurations (SM with glass coverslips and SM with transwells) were used. This indicates that the two different machine configurations provide similar data.

A comparison of the reproducibility of surfactant ingredients, non-surfactant ingredients, and surfactant based formulations and mixtures was conducted (Table 5.2.1.a, 5.2.1.b, and 5.2.1.c), and it could be seen that variability for the non-surfactant appeared slightly higher (CV non-surfactant 52.8% [two wildly disparate values only; one of 103.5% and one of 2.5%], surfactant ingredients 29.8%, and surfactant based formulations and mixtures 36.7%).

Table 5.2.1.a Surfactant based formulations and mixtures - Interlaboratory reproducibility of SM results from Bagley, Bruner et al. 1992 study. The number of replicates for each lab is unknown. N = 21 surfactant formulations.

Chemical	Conc. tested	MRD_{50} values (mg/mL)		Mean MRD_{50} (mg/mL)	SD	CV (%)
		Lab 1	Lab 2			
Surfactant Formulations						
Baby shampoo	100%	0.129	0.068	0.098	0.043	43.8
Bar soap, 10%	10%	0.063	0.059	0.061	0.003	4.4
Bath foam	100%	1.905	3.767	2.836	1.316	46.4
Bath gel/bath foam	100%	3.451	4.121	3.786	0.473	12.5
Dishwashing liquid	100%	1.528	2.158	1.843	0.446	24.2
Dishwashing liquid	100%	7.228	4.519	5.873	1.916	32.6
Ethyl acetate, 10% in Tween 80	10%	0.018	0.032	0.025	0.01	39.9
Foaming bath, 10%	10%	0.096	0.09	0.093	0.004	4.1
Hair conditioner	100%	0.003	0.002	0.002	0.001	28.3
Hair gel	100%	0.014	0.032	0.023	0.013	56.2
Hair gel	100%	0.005	0.003	0.004	0.001	40.7
Laundry detergent liquid	100%	29.309	1.419	15.364	19.721	128.4
Liquid soap	100%	1.919	1.535	1.727	0.272	15.7
Shampoo	100%	3.532	3.819	3.676	0.203	5.5
Shampoo	100%	2.716	3.811	3.264	0.774	23.7
Shampoo	100%	2.286	0.394	1.34	1.338	99.9
Shampoo	100%	4.406	0.731	2.568	2.598	101.2
Shower gel	100%	3.864	3.793	3.828	0.05	1.3
Shower gel with baby oil, 10%	10%	0.062	0.044	0.053	0.012	23.1
Shower gel, 10%	10%	0.104	0.078	0.091	0.018	20.2
Skin cleanser	100%	0.232	0.178	0.205	0.038	18.6
Mean – All Surfactant Materials						**36.7**
Median – All Surfactant Materials						**24.2**

Table 5.2.1.b Surfactant ingredients - Interlaboratory reproducibility of SM results from Bagley, Bruner et al. 1992 study. The number of replicates for each lab is unknown. N = 9 surfactant chemicals.

Chemical	Conc. tested	MRD_{50} values (mg/mL) Lab 1	Lab 2	Mean MRD_{50}	SD MRD_{50}	CV (%)
Surfactant Chemicals						
CTAB, 10%	10%	2.877	2.495	2.686	0.271	10.1
CTAC, 1%	1%	0.519	0.509	0.514	0.007	1.3
MTAB, 10%	10%	1.75	2.323	2.036	0.405	19.9
Sodium lauryl sulphate, 10%	10%	1.374	1.262	1.318	0.079	6
Sodium lauryl sulphate, 5%	5%	0.448	1.033	0.74	0.414	55.9
Triton X-100, 10%	10%	0.144	1.489	0.817	0.951	116.5
Tween 20	100%	0.031	0.033	0.032	0.001	4.2
Tween 20, 10%	10%	0.006	0.005	0.005	0.001	14.3
Tween 80, 10%	10%	0.001	0.003	0.002	0.001	39.9
Mean – All Surfactant Chemicals						**29.8**
Median – All Surfactant Chemicals						**14.3**

Table 5.2.1.c Non-surfactant ingredients – Interlaboratory reproducibility of SM results from Bagley, Bruner et al. 1992 study. The number of replicates for each lab is unknown. N = 2 non-surfactant chemicals.

Chemical	Conc. tested	MRD_{50} values (mg/mL) Lab 1	Lab 2	Mean MRD_{50}	SD MRD_{50}	CV (%)
Non-Surfactant Chemicals						
Citric acid, 18%	100%	0.087	0.564	0.325	0.337	103.5
Triethanolamine	100%	0.017	0.017	0.017	0.00	2.1
Mean – All Non-Surfactant Chemicals						**52.8**
Median – All Non-Surfactant Chemicals						**52.8**

5.2.2 EC/HO study

The EC/HO study was conducted with the CM fitted with a transwell. L929 cells were used as the target with an exposure time of 810 seconds. Four laboratories participated using a single protocol, but not enough information is available to establish how closely the participants in each laboratory adhered to the protocol. It is known that no formal training sessions were held between the laboratories. Sixty coded test materials which ranged across a wide variety of chemicals were tested. Raw data can be found in Annex H7 & H13 (Lovell, BIBRA Project No. 1367/1, Vol.1).

5.2.2.1 Reproducibility of "testable" decision for EC/HO study

One measure of reproducibility is a comparison of the somewhat subjective decision process involved in determining whether a material actually meets the criteria for compatibility with the test system. This is relatively easy to investigate for the EC/HO study since each laboratory made its own decision whether or not the chemical was compatible with the test system, i.e. was aqueous soluble and relatively non-viscous. The table below shows the results of this criterion being applied independently by each laboratory.

Table 5.2.2.1 Decisions of the laboratories involved in the EC/HO study as to whether individual test materials were compatible with testing in the CM. Data from Annex H13.

Number of labs agreeing material was "testable"	Number of test materials (% of total)
4 of 4	28 (46.6%)
3 of 4	2 (3.3%)
2 of 4	4 (6.7%)
1 of 4	10 (16.7%)
0 of 4	16 (26.7%)

It can be seen that in most of the cases (73.3%) all four labs came to the same decision, i.e. either all of the labs decided the material was "testable" or all of the labs decided it was not "testable". However, in 26.7% of the cases (16 chemicals), there was disagreement; one or more of the labs made a different decision than the remaining labs. It appears that there is some subjectivity in the "testable" or "not testable" decision.

A second question that can be asked about the reproducibility of the "testable" decision is whether one laboratory was constantly an outlier in the testable decision. In this study for the ten cases where only one laboratory concluded that the material was testable Laboratories 30 and 32 were each responsible for four of the decisions, with Laboratories 31 and 33 responsible for one each of the remaining two cases. There were two cases where three of four laboratories decided to test and one laboratory did not, in one case the outlier was Laboratory 32 and in the other case Laboratory 33. Thus it appears that Laboratory 32 was responsible for more of the "outlier" decisions than any other laboratory.

A third question that can be asked is: for situations where one or more laboratories determined that a material was not testable, did the remaining laboratories that did test the material show good or poor reproducibility? For the two chemicals (thiourea and ammonium nitrate) for which data were reported by three of the four labs, the results were split with thiourea being reproducible and ammonium nitrate variable (Table 5.2.2.2.b). For the four chemicals (cyclohexanol, l-aspartic acid, sodium perborate, and methylcyanoacetate) which were tested in only two laboratories, the results showed great variability. Although the results for L-aspartic acid were very similar between the laboratories, the results for the other three chemicals were quite different between the laboratories. These results suggest that as the disagreement among the labs as to the

testability of the chemical increases, the results that they produce are more disparate. This is good evidence that when two or more labs agreed that the chemical was not compatible, that this was the correct decision since there did appear to be problems in the testing of those chemicals.

It should be noted that none of the surfactants were determined to be untestable by any of the four laboratories.

5.2.2.2 Reproducibility of MRD_{50} values for the EC/HO study

A comparison of the MRD_{50} values for each of the 44 EC/HO test materials (16 of the 60 total materials could not be tested in the CM) which one or more of the participating laboratories tested is given in Table 5.2.2.2.a (12 surfactants) and Table 5.2.2.2.b (32 non-surfactants). These data come from the Bibra report (Lovell, BIBRA Project No. 1367/1, Vol.1). The mean, SD and CV values are based on the MRD_{50} values expressed in mg/ml. SD's and CV's are listed for the 23 non-surfactant chemicals where at least two of the four laboratories provided data. The overall average CV was 46.2% (37.0% for surfactants; 50.6% for non-surfactants). The largest discrepancies were found for situations where only two laboratories tested the materials; in three of these cases the CV's were greater than 100%. If only the 28 chemicals (11 surfactants, 17 non-surfactants) for which all four laboratories provided data are considered, the average CV drops to 38.2%.

It is likely that the relatively high variability was due to the fact that a general training session for all the labs was not held before the study was conducted. It also appears that the data produced by laboratory 32 often appeared to be at variance with the other three laboratories. Although it is tempting to reevaluate the variability only using data from the other three laboratories, this may give an erroneous impression of the reproducibility of the assay in the absence of formal interlaboratory training sessions.

Table 5.2.2.2.a Surfactant Materials - Between-laboratories reproducibility of CM results from EC/HO study. The number of replicates for each lab is unknown. N = 12 surfactant chemicals. Data from Annex H13.

Chemical	Conc. tested	MRD_{50} Values (mg/mL)				Mean MRD_{50} (mg/mL)	SD	CV (%)
		CM 30	CM 31	CM 32	CM 33			
Surfactant Chemicals								
Benzalkonium chloride	5%	1.15	1.09	0.98	1.28	1.13	0.12	11.1
Benzalkonium chloride	10%	0.26	0.47	0.38	0.44	0.39	0.09	24.2
Benzalkonium chloride [1]/[2]	1%	4.71	5.16	4.65	3.58	4.53	0.67	14.8
Cetylpyridinium bromide	10%	0.78	1.02	2.34	0.89	1.26	0.73	58.2
Cetylpyridinium bromide	6%	0.6	1.35	0.44	1.11	0.87	0.43	48.8
Cetylpyridinium bromide	0.1%	48.19	102.33	7.76	180.3	84.65	74.62	88.1
Polyethylene glycol 400	100%	*	*	*	363.92	*	*	*
Sodium lauryl sulfate	15%	0.62	0.6	0.51	0.74	0.62	0.1	15.5
Sodium lauryl sulfate	3%	2.71	3.04	3.74	3.64	3.28	0.49	15
Triton X-100	10%	1.61	1.96	1.5	2.22	1.82	0.33	18
Triton X-100 [1]/[2]	5%	1.9	3.39	5.09	2.53	3.23	1.39	43
Tween 20	100%	1.52	5.53	4.98	1.06	3.27	2.31	70.5
Mean – Surfactant Chemicals								**37.0**
Median – Surfactant Chemicals								**24.2**

* Participating laboratory did not test the chemical because it determined that chemical was not compatible with the test system.

Table 5.2.2.2.b Non-surfactant materials - Between-laboratories reproducibility of CM results from EC/HO study. The number of replicates for each lab is unknown. N = 48 non-surfactant chemicals. Data from Annex H13.

Chemical	Conc. tested	MRD_{50} Values (mg/mL)				Mean MRD_{50} (mg/mL)	SD	CV (%)
		CM 30	CM 31	CM 32	CM 33			
Non-Surfactant Chemicals								
1-Naphthalene acetic acid	100%	12.11	*	*	*	*	*	*
1-Naphthalene acetic acid	100%	*	*	*	*	*	*	*
2,2-Dimethylbutanoic acid	100%	*	*	*	*	*	*	*
2,5-Dimethylohexanediol	100%	76.21	155.96	6.21	156.31	98.67	72.25	73.2
2,6-Dichlorobenzoyl chloride	100%	*	*	*	*	*	*	*
2-Ethyl-1-hexanol	100%	*	*	*	*	*	*	*
4-Carboxybenzaldehyde	100%	*	*	*	*	*	*	*
Acetone	100%	153.82	140.28	139	162.18	148.82	11.15	7.5
Ammonium nitrate	100%	40.27	145.55	27.99	*	71.27	64.62	90.7
Benzoyl-L-tartaric acid	100%	0.81	*	*	*	*	*	*
Captan 90 concentrate	100%	*	*	*	*	*	*	*
Chlorhexidine	100%	*	*	*	*	*	*	*
Cyclohexanol	100%	15.49	*	0.58	*	8.03	10.5	131.3
Dibenzyl phosphate	100%	0.75	*	*	*	*	*	*
Ethanol	100%	97.05	117.49	123.03	110.41	111.99	11.22	10

Ethyl acetate	100%	*	53.7	*	*	*	*	*
Ethyl trimethyl acetate	100%	*	*	*	*	*	*	*
Ethyl-2-methylacetoacetate	100%	*	*	0.4	*	*	*	*
Fomesafen	100%	*	*	*	*	*	*	*
Gammabutyrolactone	100%	79.98	114.82	0.91	179.47	93.79	74.39	79.3
Glycerol	100%	121.62	180.72	8.26	208.93	129.88	88.87	68.4
Imidazole	100%	22.75	23.07	0.18	48.75	23.69	19.85	83.8
Isobutanol	100%	28.84	28.64	22.54	31.62	27.91	3.83	13.7
Isopropanol	100%	83.18	91.2	87.1	143.55	101.26	28.39	28
L-Aspartic acid	100%	1.11	1.17	*	*	1.14	0.04	3.6
Maneb	100%	*	*	*	*	*	*	*
Methyl acetate	100%	61.09	91.83	116.14	109.65	94.68	24.64	26
Methyl cyanoacetate	100%	42.95	*	0.13	*	21.54	30.28	140.5
Methyl ethyl ketone	100%	55.72	50.47	78.16	47.97	58.08	13.77	23.7
Methyl isobutyl ketone	100%	*	*	0.81	*	*	*	*
Methylcyclopentane	100%	*	*	*	*	*	*	*
n-Butyl acetate	100%	*	*	*	*	*	*	*
n-Hexanol	100%	*	*	*	*	*	*	*
n-Octanol	100%	*	*	*	*	*	*	*
Parafluoraniline	100%	*	*	3.48	*	*	*	*
Potassium cyanate	100%	28.18	36.06	9.4	50.82	31.11	17.25	55.4
Promethazine HCl	100%	1.35	1.48	0.81	1.45	1.27	0.31	24.4
Pyridine	100%	1.54	29.99	15.92	31.48	19.73	14.01	71
Quniacrine	100%	*	*	1.08	*	1.08	*	*
Sodium hydroxide	10%	2.28	1.6	2.67	2.49	2.26	0.47	20.8
Sodium hydroxide	1%	28.18	16.22	32.36	31.41	27.04	7.48	27.5
Sodium oxalate	100%	*	*	*	*	*	*	*
Sodium perborate, 4H20	100%	0.11	*	*	3.27	1.69	2.24	132.6
Tetraaminopyrimidine sulfate	100%	1.05	*	*	*	*	*	*
Thiourea	100%	50.12	50.93	*	47.97	49.68	1.53	3.1
Toluene	100%	*	*	*	*	*	*	*
Trichloroacetic acid	30%	1.69	2.47	0.81	2.2	1.79	0.73	40.7
Trichloroacetic acid	3%	13.9	13.8	16.29	16.11	15.03	1.36	9
Mean – Non-Surfactant Chemicals								**50.6**
Median – Non-Surfactant Chemicals								**28.0**
Mean when all four labs tested material – All Materials								**38.2**

* Participating laboratory did not test the chemical because it determined that chemical was not compatible with the test system.

In addition to the above table, an extensive analysis of the reproducibility and predictivity of the assays in the EC/HO study, not presented in the Balls, Botham et al. 1995 manuscript on the EC/HO study, was prepared by BIBRA International as BIBRA

Project No. 1367. This analysis consisted of graphical representations of the log transformed scores for each lab for each test material (Figure 5.2.2.2.a), a plot of residuals for each lab for each chemical (Figure 5.2.2.2.b), and a correlation matrix for all four laboratories with each other and with Draize MMAS scores (Table 5.2.2.2.c).

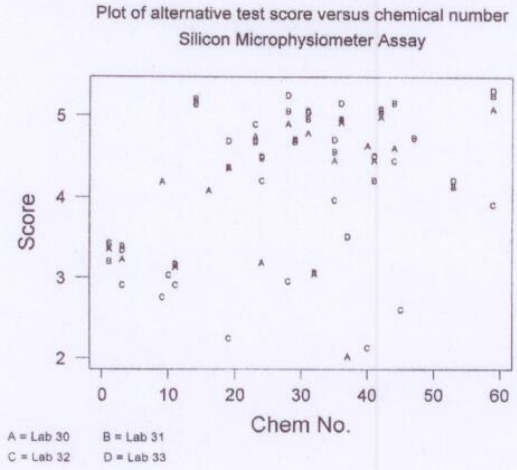


Figure 5.2.2.2.a Plot of CM test scores (log MRD_{50}) versus individual chemicals for the EC/HO study. From BIBRA project report 1367.

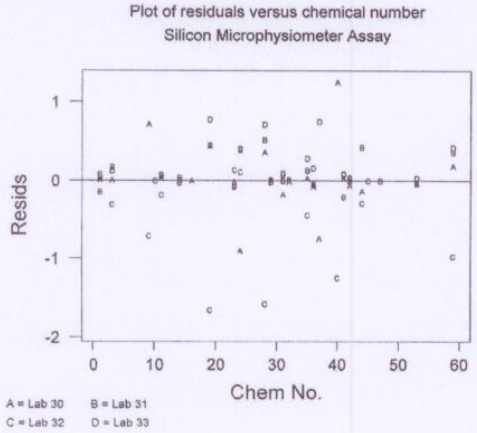


Figure 5.2.2.2.b Plot of residuals of CM test scores versus individual chemicals for the EC/HO study. From BIBRA project report 1367.

Table 5.2.2.2.c Correlation matrix of alternative CM test scores from individual laboratories for the EC/HO study. From BIBRA project report 1367.

Correlation Matrix of alternative test scores and MMAS
Silicon Microphysiometer Assay

	MMAS	Lab 30	Lab 31	Lab 32	Lab 33
MMAS	1.000				
Lab 30	-0.357	1.000			
Lab 31	-0.591	0.901	1.000		
Lab 32	-0.328	0.427	0.569	1.000	
Lab 33	-0.577	0.875	0.986	0.507	1.000

The Figures 5.2.2.2.a and 5.2.2.2.b, and Table 5.2.2.2.c indicate that the results from three of the labs (30, 31, and 33) correlate quite well, while the results from lab 32 often appear as outliers. This is similar to the conclusions from the "testability" decisions where lab 32 appeared to be responsible for more of the outlier decisions than any other laboratory. There is not enough information available at this time to determine what the reasons for variability of the MRD_{50} results are. Table 5.2.2.2.c should be viewed with the understanding that even though the results between two laboratories have a high linear correlation, the actual values obtained can be quite different since one laboratory may be consistently higher or lower than the other laboratory.

5.2.2.3 Reproducibility of predicted hazard classifications for the EC/HO study

A comparison of the between laboratories reproducibility of the prediction of hazard classifications is given in this section. Since none of the formal studies of the CM reported on in this BRD had predetermined prediction models for hazard classifications (although several did for Draize scores), the following analyses are based on prediction models derived during the construction of this BRD and presented in Chapter 6 – Predictive Capacity. Specifically these analyses of the EC/HO study are based on the prediction models proposed in Section 6.1.3.1.

Tables 5.2.2.3.a and 5.2.2.3.b. present the predicted EU, GHS and EPA classifications predicted for the surfactant and non-surfactant materials, respectively from the MRD_{50} values produced by each of the four participating laboratories. These

predictions were then consolidated into summary tables which are Tables 5.2.2.3.c and d for the surfactants and non-surfactant materials, respectively.

Table 5.2.2.3.c shows that for the surfactant materials where all four laboratories tested the materials (all but one of the cases) that 6 of the 11 materials were predicted to be the same classification, 3 of the 11 materials were predicted identically by 3 of the 4 labs, and 2 of the materials were had similar predictions for between less than three of the labs.

Table 5.2.2.3.d shows that for the non-surfactant materials where all four laboratories tested the materials that 9 of 17 materials were predicted the same by all four labs. Five materials had agreement between only 3 of the 4 labs and 3 of the 17 materials had agreement between less than 3 of the labs.

For the two non-surfactant materials where only three of the labs tested the materials three labs agreed on one and only two labs agreed on the other. If only two labs tested the materials, then both agreed for one material and both disagreed for the remaining three materials.

It appears from the above data that as fewer labs decided that a material was not testable under the constraints of the protocol, the reproducibility of the hazard predictions became worse.

Table 5.2.2.3.a Surfactant Materials - EU, GHS, and EPA classifications based on Cytosensor MRD_{50} values from EC/HO study. Cut-off values from Figures 6.1.3.1.a, 6.1.3.1.b, and 6.1.3.1.c were used. The number of replicates for each lab is unknown. N = 12 surfactant materials.

Chemical	Conc. tested	EU				GHS				EPA			
		CM 30	CM 31	CM 32	CM 33	CM 30	CM 31	CM 32	CM 33	CM 30	CM 31	CM 32	CM 33
Benzalkonium chloride	5%	R41	R41	R41	R41	1	1	1	1	I	I	I	I
Benzalkonium chloride	10%	R41	R41	R41	R41	1	1	1	1	I	I	I	I
Benzalkonium chloride [1]/[2]	1%	R36	R36	R36	R36	2A or 2B	2A or 2B	2A or 2B	2A or 2B	II or III	II or III	II or III	II or III
Cetylpyridinium bromide	10%	R41	R41	R36	R41	1	1	2A or 2B	1	I	I	II or III	I
Cetylpyridinium bromide	6%	R41	R41	R41	R41	1	1	1	1	I	I	I	I
Cetylpyridinium bromide	0%	R36	NL	R36	NL	2A or 2B	NL	2A or 2B	NL	II or III	IV	II or III	IV
Polyethylene glycol 400	100%	*	*	*	NL	*	*	*	NL	*	*	*	IV
Sodium lauryl sulfate	15%	R41	R41	R41	R41	1	1	1	1	I	I	I	I
Sodium lauryl sulfate	3%	R36	R36	R36	R36	2A or 2B	2A or 2B	2A or 2B	2A or 2B	II or III	II or III	II or III	II or III
Triton X-100	10%	R41	R41	R41	R36	1	1	1	2A or 2B	I	I	I	II or III
Triton X-100 [1]/[2]	5%	R41	R36	R36	R36	1	2A or 2B	2A or 2B	2A or 2B	I	II or III	II or III	II or III
Tween 20	100%	R41	R36	R36	R41	1	2A or 2B	2A or 2B	1	I	II or III	II or III	I

* = not tested

Table 5.2.2.3.b Non-surfactant Materials – EU, GHS, and EPA classifications based on Cytosensor MRD_{50} values from EC/HO study. Cut-off values from Figures 6.1.3.1.a, 6.1.3.1.b, and 6.1.3.1.c were used. The number of replicates for each lab is unknown. N = 48 non-surfactant materials.

Chemical	Conc. tested	EU				GHS				EPA			
		CM 30	CM 31	CM 32	CM 33	CM 30	CM 31	CM 32	CM 33	CM 30	CM 31	CM 32	CM 33
1-Naphthalene acetic acid	100%	R36	*	*	*	2A or 2B	*	*	*	II or III	*	*	*
1-Naphthalene acetic acid	100%	*	*	*	*	*	*	*	*	*	*	*	*
2,2-Dimethylbutanoic acid	100%	*	*	*	*	*	*	*	*	*	*	*	*
2,5-Dimethylohexanediol	100%	R36	NL	R36	NL	2A or 2B	NL	2A or 2B	NL	II or III	IV	II or III	IV
2,6-Dichlorobenzoyl chloride	100%	*	*	*	*	*	*	*	*	*	*	*	*
2-Ethyl-1-hexanol	100%	*	*	*	*	*	*	*	*	*	*	*	*
4-Carboxybenzaldehyde	100%	*	*	*	*	*	*	*	*	*	*	*	*
Acetone	100%	NL	NL	NL	NL	NL	NL	NL	NL	IV	IV	IV	IV
Ammonium nitrate	100%	R36	NL	R36	*	2A or 2B	NL	2A or 2B	*	II or III	IV	II or III	*
Benzoyl-L-tartaric acid	100%	R41	*	*	*	1	*	*	*	I	*	*	*
Captan 90 concentrate	100%	*	*	*	*	*	*	*	*	*	*	*	*
Chlorhexidine	100%	*	*	*	*	*	*	*	*	*	*	*	*
Cyclohexanol	100%	R36	*	R41	*	2A or 2B	*	1	*	II or III	*	I	*
Dibenzyl phosphate	100%	R41	*	*	*	1	*	*	*	I	*	*	*
Ethanol	100%	NL	NL	NL	NL	NL	NL	NL	NL	IV	IV	IV	IV
Ethyl acetate	100%	*	R36	*	*	*	2A or 2B	*	*	*	II or III	*	*
Ethyl trimethyl acetate	100%	*	*	*	*	*	*	*	*	*	*	*	*
Ethyl-2-methylacetoacetate	100%	*	*	R41	*	*	*	1	*	*	*	I	*
Fomesafen	100%	*	*	*	*	*	*	*	*	*	*	*	*
Gammabutyrolactone	100%	R36	NL	R41	NL	2A or 2B	NL	1	NL	II or III	IV	I	IV
Glycerol	100%	NL	NL	R36	NL	NL	NL	2A or 2B	NL	IV	IV	II or III	IV
Imidazole	100%	R36	R36	R41	R36	2A or 2B	2A or 2B	1	2A or 2B	II or III	II or III	I	II or III
Isobutanol	100%	R36	R36	R36	R36	2A or 2B	2A or 2B	2A or 2B	2A or 2B	II or III	II or III	II or III	II or III
Isopropanol	100%	NL	NL	NL	NL	NL	NL	NL	NL	IV	IV	IV	IV
L-Aspartic acid	100%	R41	R41	*	*	1	1	*	*	I	I	*	*
Maneb	100%	*	*	*	*	*	*	*	*	*	*	*	*
Methyl acetate	100%	R36	NL	NL	NL	2A or 2B	NL	NL	NL	II or III	IV	IV	IV
Methyl cyanoacetate	100%	R36	*	R41	*	2A or 2B	*	1	*	II or III	*	I	*
Methyl ethyl ketone	100%	R36	R36	R36	R36	2A or 2B	2A or 2B	2A or 2B	2A or 2B	II or III	II or III	II or III	II or III
Methyl isobutyl ketone	100%	*	*	R41	*	*	*	1	*	*	*	I	*
Methylcyclopentane	100%	*	*	*	*	*	*	*	*	*	*	*	*

n-Butyl acetate	100%	*	*	*	*	*	*	*	*	*	*	*	*
n-Hexanol	100%	*	*	*	*	*	*	*	*	*	*	*	*
n-Octanol	100%	*	*	*	*	*	*	*	*	*	*	*	*
Parafluoraniline	100%	*	*	R36	*	*	*	2A or 2B	*	*	*	II or III	*
Potassium cyanate	100%	R36	R36	R36	R36	2A or 2B	2A or 2B	2A or 2B	2A or 2B	II or III	II or III	II or III	II or III
Promethazine HCl	100%	R41	R41	R41	R41	1	1	1	1	I	I	I	I
Pyridine	100%	R41	R36	R36	R36	1	2A or 2B	2A or 2B	2A or 2B	I	II or III	II or III	II or III
Quniacrine	100%	*	*	R41	*	*	*	1	*	*	*	I	*
Sodium hydroxide	10%	R36	R41	R36	R36	2A or 2B	1	2A or 2B	2A or 2B	II or III	I	II or III	II or III
Sodium hydroxide	1%	R36	R36	R36	R36	2A or 2B	2A or 2B	2A or 2B	2A or 2B	II or III	II or III	II or III	II or III
Sodium oxalate	100%	*	*	*	*	*	*	*	*	*	*	*	*
Sodium perborate, 4H20	100%	R41	*	*	R36	1	*	*	2A or 2B	I	*	*	II or III
Tetraaminopyrimidine sulfate	100%	R41	*	*	*	1	*	*	*	I	*	*	*
Thiourea	100%	R36	R36	*	R36	2A or 2B	2A or 2B	*	2A or 2B	II or III	II or III	*	II or III
Toluene	100%	*	*	*	*	*	*	*	*	*	*	*	*
Trichloroacetic acid	30%	R41	R36	R41	R36	1	2A or 2B	1	2A or 2B	I	II or III	I	II or III
Trichloroacetic acid	3%	R36	R36	R36	R36	2A or 2B	2A or 2B	2A or 2B	2A or 2B	II or III	II or III	II or III	II or III

* = not tested

Table 5.2.2.3.c Surfactant Materials – Agreement table for EU, GHS, and EPA classifications based on Cytosensor MRD_{50} values for the EC/HO study.

Where 4 labs tested the material			
Agreement	EU	GHS	EPA
4 labs	6	6	6
3 labs	3	3	3
<3 labs	2	2	2

Table 5.2.2.3.d Non-Surfactant Materials – Agreement table for EU, GHS, and EPA classifications based on Cytosensor MRD_{50} values for the EC/HO study.

Where 4 labs tested the material			
Agreement	EU	GHS	EPA
4 labs	9	9	9
3 labs	5	5	5
<3 labs	3	3	3
Where 3 labs tested the material			
Agreement	EU	GHS	EPA
3 labs	1	1	1
2 labs	1	1	1
<2 labs	0	0	0
Where 2 labs tested the material			
Agreement	EU	GHS	EPA
Both agree	1	1	1
Both disagree	3	3	3

5.2.3 COLIPA study

The COLIPA study (Brantom, Bruner et al. 1997; Harbell, Osborne et al. 1999) was conducted with the CM which utilizes a transwell. L929 cells were used as the target with an exposure time of 810 seconds. The two instruments used in the study were essentially identical; differing only in that one instrument had two 4-channel modules and the other one 4-channel module. Both laboratories participated using a single protocol (Annex A), but not enough information is available to establish how closely the participants in each laboratory adhered to the protocol. Each laboratory was asked to conduct the study according to the "spirit of GLP". The results from each laboratory were subjected to a quality audit by the QAU from BIBRA, but the individual laboratories did not document the extent of QA that was applied within their own laboratories. It is known that the protocol and potential problem areas were discussed between the study directors before the start of the testing phase. Fifty-five coded test materials (23 chemicals, 32 products) which were representative of substances commonly used in the cosmetics industry were tested.

5.2.3.1 Reproducibility of "testable" decision for COLIPA study

Each laboratory made its own decision whether or not the chemical was compatible with the test system, i.e. formed a true solution in the culture medium. It was reported (Harbell, Osborne et al. 1999) that six of the 55 samples formed microsuspensions which were not acceptable for testing under the strict requirements of the protocol for this study; however, the authors stated that microsuspensions were normally tested under current practice. The table below shows the results of this criterion being applied independently by each laboratory.

Table 5.2.3.1 Decisions of the laboratories involved in the COLIPA study as to whether individual test materials were compatible with testing in the CM. Data from Annexes F13 & H29.

Number of labs agreeing material was "testable"	Number of test materials (% of total)
2 of 2	26 (47.2%)
1 of 2	3 (5.5%)
0 of 2	26 (47.2%)

Similar to the results of the EC/HO study, in most of the cases (94.4%) both labs came to the same decision, i.e. either both of the labs decided the material was "testable" or both of the labs decided it was not "testable". In only 5.5% of the cases (3 chemicals out of 55) was there disagreement. This study showed less subjectivity in the "testable" or "not testable" decision then the EC/HO study, perhaps because of the clear protocol prohibition against testing any material which was not in a single phase at the highest tested concentration. The three discrepant chemicals were: 1) cetylpyridinium bromide 6% which was tested by Company # 4 but not by Company # 5 (cetylpyridinum bromide 10% was considered unsuitable for testing by both labs), 2) sodium lauryl sulphate 30% which was tested by Company # 4 but not by Company # 5 (sodium lauryl sulfate 10% was tested by both labs), and 3) methyl ethyl ketone which again was tested by Company # 4 and not by Company # 5.

5.2.3.2 Reproducibility of MRD_{50} values for the COLIPA study

A comparison of the MRD_{50} values for each of the 26 COLIPA test materials which one or more of the participating laboratories tested is given in Tables 5.2.3.2.a, 5.2.3.2.b, and 5.2.3.2.c. The mean, SD and CV values are based on the MRD_{50} values expressed in mg/ml. SD's and CV's are only listed for the 26 chemicals where both participating laboratories provided data.

The mean CV for the surfactant materials was 23.3%, for the surfactant based formulations and mixtures was 16.5%, and for the non-surfactant materials was 32.5%. The mean CV for the non-surfactants was highly influenced by the 106.9% CV of 10% NaOH, as might be expected from the dilution protocol which is used to test the materials. The overall average CV was 24.7%.

Table 5.2.3.2.a Surfactant Materials - Between-laboratories reproducibility of Cytosensor Microphysiometer results from COLIPA study. N = 13 surfactant chemicals. Data from Annexes F13 & H29.

Chemical	Conc. tested	MRD_{50} Values (mg/mL)		Number of Replicates		Mean MRD_{50} (mg/mL)	SD	CV (%)
		Company # 4	Company # 5	Company # 4	Company # 5			
Surfactant Chemicals								
Benzalkonium chloride 1%	1%	4.11	4.33	3	3	4.22	0.16	3.7
Benzalkonium chloride 10%	10%	0.32	0.31	3	3	0.31	0.01	3.2
Benzalkonium chloride 5%	5%	0.81	1.38	3	3	1.1	0.4	36.7
Cetylpyridinium bromide 10%	10%	*	*	-	-			
Cetylpyridinium bromide 6%	6%	1.36	*	3	-	1.36		
Polyethylene glycol 400	100%	296.5	316.23	3	2	306.36	13.95	4.6
SLS 15%	15%	0.52	0.51	3	3	0.51	0.01	1
SLS 3%	3%	3.23	2.78	3	3	3	0.32	10.6
SLS 30%	30%	0.31	*	3	-	0.31		
Triton X-100 1%	1%	21.17	16.79	3	3	18.98	3.1	16.3
Triton X-100 10%	10%	2.47	1.24	3	3	1.85	0.87	46.8
Triton X-100 5%	5%	4.66	2.42	3	3	3.54	1.58	44.7
Tween 20	100%	9.5	3.49	3	3	6.5	4.25	65.4
Mean – Surfactant Chemicals								**23.3**
Median – Surfactant Chemicals								**13.5**

* - Participating laboratory did not test the chemical because it determined that chemical was not compatible with the test system.

Table 5.2.3.2.b Surfactant based formulations and mixtures - Between-laboratories reproducibility of Cytosensor Microphysiometer results from COLIPA study. N = 22 surfactant formulations. Data from Annexes F13 & H29.

Chemical	Conc. tested	MRD_{50} Values (mg/mL)		Number of Replicates		Mean MRD_{50} (mg/mL)	SD	CV (%)
		Company # 4	Company # 5	Company # 4	Company # 5			
Surfactant Formulations								
Cleansing foam III	100%	*	*	-	-	-	-	-
Emulsion antiperspirant	100%	*	*	-	-	-	-	-
Eye make-up remover	100%	87.77	99.31	3	3	93.54	8.16	8.7
Gel cleaner	100%	5.68	5.47	3	3	5.58	0.15	2.6
Hair conditioner	100%	*	*	-	-	-	-	-
Hair dye base F#1	100%	*	*	-	-	-	-	-
Hair dye base form #3	100%	*	*	-	-	-	-	-
Hand cleaner	100%	*	*	-	-	-	-	-
Hand soap	100%	*	*	-	-	-	-	-
Hydrophilic ointment	100%	*	*	-	-	-	-	-
Liquid soap #1	100%	0.88	0.68	3	3	0.78	0.14	18.5
Moisturiser with sunscreen	100%	*	*	-	-	-	-	-
Perfumed skin lotion	100%	*	*	-	-	-	-	-
Polishing scrub	100%	*	*	-	-	-	-	-
Pump Deodorant	5%	19.35	47.74	3	3	33.54	20.08	59.9
Shampoo – baby	100%	2.51	2.15	3	3	2.33	0.25	10.8
Shampoo #1 normal	100%	0.75	0.72	3	3	0.74	0.02	2.2
Shampoo 2-in-1	100%	*	*	-	-	-	-	-
Shampoo antidandruff	100%	*	*	-	-	-	-	-
Shower gel	100%	*	*	-	-	-	-	-
Skin cleaner	100%	0.63	0.76	3	3	0.7	0.09	13
Sunscreen SPF 15	100%	*	*	-	-	-	-	-
Mean – Surfactant Formulations							**16.5**	
Median – Surfactant Formulations							**10.8**	

* - Participating laboratory did not test the chemical because it determined that chemical was not compatible with the test system.

Table 5.2.3.2.c Non-Surfactants, ingredients, and mixtures - Between-laboratories reproducibility of Cytosensor Microphysiometer results from COLIPA study. N = 10 non-surfactant chemicals and N = 10 non-surfactant formulations. Data from Annexes F13 & H29.

Chemical	Conc. tested	MRD_{50} Values (mg/mL)		Number of Replicates		Mean MRD_{50} (mg/mL)	SD	CV (%)
		MA	CT AB	MA	CT AB			
Non-Surfactant Chemicals								
Ethyl acetate	100%	*	*	-	-			
Glycerol	100%	214.83	208.7	3	2	211.77	4.34	2
Imidazole	100%	18.84	26.03	3	3	22.43	5.09	22.7
Isopropanol	100%	52.59	124.51	3	3	88.55	50.86	57.4
Methyl ethyl ketone	1%	54.18	*	3	-	54.18		
n-Butyl acetate	100%	*	*	-	-			
Propylene glycol	100%	265.07	218.86	3	3	241.97	32.67	13.5
Sodium hydroxide 1%	1%	9.09	13.59	3	3	11.34	3.19	28.1
Sodium hydroxide 10%	10%	4.33	0.6	3	3	2.47	2.64	106.9
Trichloroacetic acid 30%	30%	1.12	1.24	3	3	1.18	0.09	7.3
Mean – Non-Surfactant Chemicals								**34.0**
Median – Non-Surfactant Chemicals								**22.7**
Non-Surfactant Formulations								
Blush	100%	*	*	-	-			
Cologne	100%	*	*	-	-			
Eye liner	100%	*	*	-	-			
Eye shadow	100%	*	*	-	-			
Hair dye base form #2	100%	*	*	-	-			
Hair styling lotion	100%	164.82	292.01	3	3	228.41	89.94	39.4
Mascara	100%	*	*	-	-			
Mouthwash	100%	37.84	46.85	3	3	42.35	6.37	15
Sunscreen lotion	10%	*	*	-	-			
Toothpaste	100%	*	*	-	-			
Mean – Non-Surfactant Formulations								**27.2**
Median – Non-Surfactant Formulations								**27.2**
Mean – All Non-Surfactant Materials								**32.5**
Median – All Non-Surfactant Materials								**22.7**

* - Participating laboratory did not test the chemical because it determined that chemical was not compatible with the test system.

5.2.3.3 Reproducibility of predicted hazard classifications for the COLIPA study

A comparison of the between laboratories reproducibility of the prediction of hazard classifications is given in this section. Since none of the formal studies of the CM reported on in this BRD had predetermined prediction models for hazard classifications (several did for Draize scores), the following analyses are based on prediction models derived during the construction of this BRD and presented in Chapter 6 – Predictive Capacity. Specifically these analyses of the COLIPA study are based on the prediction models proposed in Section 6.1.3.3.

Tables 5.2.3.3.a, b and c. present the predicted EU, GHS and EPA classifications predicted for the surfactant materials, surfactant-based formulations and non-surfactant materials, respectively from the MRD_{50} values produced by each of the two participating laboratories. These predictions were then consolidated into summary tables which are Tables 5.2.3.3.d, 5.2.3.3.e, and 5.2.3.3.f for the surfactant materials, surfactant-based formulations and non-surfactant materials, respectively.

Table 5.2.3.3.d shows that for the surfactant materials where both laboratories tested the materials that 90% (9 of the 10) materials were predicted to be the same classification for all three classification systems.

Table 5.2.3.3.e shows that for the surfactant-based formulations where both laboratories tested the materials that 100% (7 of 7) materials were predicted the same both labs.

For the 9 non-surfactant materials where both of the labs tested the materials (Table 5.2.3.3.f), 76.7% (7of 9 materials) were predicted to be the same hazard classification.

Table 5.2.3.3.a Surfactant Materials - Between-laboratories reproducibility of Cytosensor Microphysiometer hazard classifications from COLIPA study. Cut-off values are based on Figures 6.1.3.3.d, 6.1.3.3.e, and 6.1.3.3.f. N = 14 surfactant materials.

Chemical	Conc. tested	EU		GHS		EPA	
		Company # 4	Company # 5	Company # 4	Company # 5	Company # 4	Company # 5
Benzalkonium chloride 1%	1%	R36	R36	2A or 2B	2A or 2B	II or III	II or III
Benzalkonium chloride 10%	10%	R41	R41	1	1	I	I
Benzalkonium chloride 5%	5%	R41	R41	1	1	I	I
Cetylpyridinium bromide 10%	10%	*	*	*	*	*	*
Cetylpyridinium bromide 6%	6%	R41	*	1	*	I	*
Polyethylene glycol 400	100%	NL	NL	NL	NL	IV	IV
SLS 15%	15%	R41	R41	1	1	I	I
SLS 3%	3%	R36	R36	2A or 2B	2A or 2B	II or III	II or III
SLS 30%	30%	R41	*	1	*	I	*
Triton X-100 1%	1%	NL	NL	NL	NL	II or III	II or III
Triton X-100 10%	10%	R36	R41	2A or 2B	1	II or III	I
Triton X-100 5%	5%	R36	R36	2A or 2B	2A or 2B	II or III	II or III
Tween 20	100%	R36	R36	2A or 2B	2A or 2B	II or III	II or III

* - Participating laboratory did not test the chemical because it determined that chemical was not compatible with the test system.

Table 5.2.3.3.b Surfactant based formulations and mixtures - Between-laboratories reproducibility of Cytosensor Microphysiometer hazard classifications from COLIPA study. Cut-off values are based on Figures 6.1.3.3.d, 6.1.3.3.e, and 6.1.3.3.f. N = 22 surfactant based formulations.

Chemical	Conc. tested	EU		GHS		EPA	
		MA	CT AB	MA	CT AB	MA	CT AB
Cleansing foam III	100%	*	*	*	*	*	*
Emulsion antiperspirant	100%	*	*	*	*	*	*
Eye make-up remover	100%	NL	NL	NL	NL	IV	IV
Gel cleaner	100%	R36	R36	2A or 2B	2A or 2B	II or III	II or III
Hair conditioner	100%	*	*	*	*	*	*
Hair dye base F#1	100%	*	*	*	*	*	*
Hair dye base form #3	100%	*	*	*	*	*	*
Hand cleaner	100%	*	*	*	*	*	*
Hand soap	100%	*	*	*	*	*	*
Hydrophilic ointment	100%	*	*	*	*	*	*
Liquid soap #1	100%	R41	R41	1	1	I	I
Moisturiser with sunscreen	100%	*	*	*	*	*	*
Perfumed skin lotion	100%	*	*	*	*	*	*
Polishing scrub	100%	*	*				
Pump Deodorant	5%	NL	NL	NL	NL	II or III	II or III
Shampoo – baby	100%	R36	R36	2A or 2B	2A or 2B	II or III	II or III
Shampoo #1 normal	100%	R41	R41	1	1	I	I
Shampoo 2-in-1	100%	*	*	*	*	*	*
Shampoo antidandruff	100%	*	*	*	*	*	*
Shower gel	100%	*	*	*	*	*	*
Skin cleaner	100%	R41	R41	1	1	I	I
Sunscreen SPF 15	100%	*	*	*	*	*	*

* - Participating laboratory did not test the chemical because it determined that chemical was not compatible with the test system.

Table 5.2.3.3.c Non-Surfactants, ingredients, and mixtures - Between-laboratories reproducibility of Cytosensor Microphysiometer hazard classifications from COLIPA study. Cut-off values are based on Figures 6.1.3.3.a, 6.1.3.3.b, and 6.1.3.3.c. N = 10 surfactant chemicals and 10 surfactant materials.

Chemical	Conc. tested	EU		GHS		EPA	
		Company # 4	Company # 5	Company # 4	Company # 5	Company # 4	Company # 5
Surfactant Chemicals							
Ethyl acetate	100%	*	*	*	*	*	*
Glycerol	100%	NL	NL	NL	NL	IV	IV
Imidazole	100%	R36	R36	2A or 2B	2A or 2B	II or III	II or III
Isopropanol	100%	R36	NL	2A or 2B	NL	II or III	IV
Methyl ethyl ketone	1%	R36	*	2A or 2B	*	II or III	*
n-Butyl acetate	100%	*	*	*	*	*	*
Propylene glycol	100%	NL	NL	NL	NL	IV	IV
Sodium hydroxide 1%	1%	R36	R36	2A or 2B	2A or 2B	II or III	II or III
Sodium hydroxide 10%	10%	R36	R41	2A or 2B	1	II or III	I
Trichloroacetic acid 30%	30%	R41	R41	1	1	I	I
Surfactant Materials							
Blush	100%	*	*	*	*	*	*
Cologne	100%	*	*	*	*	*	*
Eye liner	100%	*	*	*	*	*	*
Eye shadow	100%	*	*	*	*	*	*
Hair dye base form #2	100%	*	*	*	*	*	*
Hair styling lotion	100%	NL	NL	NL	NL	IV	IV
Mascara	100%	*	*	*	*	*	*
Mouthwash	100%	R36	R36	2A or 2B	2A or 2B	II or III	II or III
Sunscreen lotion	10%	*	*	*	*	*	*
Toothpaste	100%	*	*	*	*	*	*

Table 5.2.3.3.d Surfactant Materials - Between-laboratories reproducibility of Cytosensor Microphysiometer hazard classifications agreement from COLIPA study.

Where 2 labs tested the material			
Agreement	EU	GHS	EPA
Both agree	9	9	9
Both disagree	1	1	1

Table 5.2.3.3.e Surfactant based formulations and mixtures - Between-laboratories reproducibility of Cytosensor Microphysiometer hazard classifications agreement from COLIPA study.

Where 2 labs tested the material			
Agreement	EU	GHS	EPA
Both agree	7	7	7
Both disagree	0	0	0

Table 5.2.3.3.f Non-Surfactants, ingredients, and mixtures - Between-laboratories reproducibility of Cytosensor Microphysiometer hazard classifications agreement from COLIPA study.

Where 2 labs tested the material			
Agreement	EU	GHS	EPA
Both agree	7	7	7
Both disagree	2	2	2

5.2.4 Analysis of materials common to both the EC/HO and COLIPA studies

The EC/HO and the COLIPA studies shared 20 test materials for which data were produced by all six (5 unique) laboratories (4 EC/HO laboratories and 2 COLIPA laboratories with one laboratory which participated in both studies). This allows between-laboratory reproducibility over a longer time period (~21 months). The general reproducibility of these studies was presented by Harbell, Osborne et al. 1999 in a tabular format shown here as Table 5.2.4.a. The correlation coefficients presented only represent results derived from fourteen materials where data were obtained from all 6 laboratories (see Tables 5.2.4.b and 5.2.4.c).

Table 5.2.4.a Between laboratories reproducibility correlation coefficients for 14 common test materials tested in the EC/HO (30-33) and COLIPA (27-28) studies. Lab 27 and 31 are the same laboratory (Harbell, Osborne et al. 1999).

Lab	27	28	30	31	32	33	
27		0.96	0.93	0.97	0.68	0.93	Pearson correlation
28	0.94		0.96	0.99	0.59	0.95	
30	0.88	0.86		0.97	0.68	0.99	
31	0.95	0.96	0.91		0.57	0.96	
32	0.71	0.62	0.71	0.69		0.61	
33	0.88	0.85	0.97	0.89	0.60		
Spearman correlation							

It can be seen that in general there is a very good correlation (around 0.9 or greater) between at least four of the laboratories. Only one lab (32) appears to be a slight outlier with correlation coefficients with the other labs that range between 0.57 and 0.71.

Tables 5.2.4.b and 5.2.4.c show a comparison of results between the six laboratories (five unique) for the 20 materials common to the COLIPA and EC/HO studies. It can be seen that there were only 14 materials where data were obtained from all six laboratories. For the surfactants (Table 5.2.4.b), one difference between the studies was that neither lab in the COLIPA study determined that 10% cetylpyridinium bromide met the criteria for testing, whereas all four labs did in the EC/HO study. This is probably due to the fact that the protocol for the COLIPA study was more descriptive about the qualifying criteria used. With a slightly more dilute mixture of cetylpyridinium bromide (6%), all but

one laboratory determined that it could be tested. The CV's for between-laboratory reproducibility ranged from 1.2% to 46.6%.

For the non-surfactant materials (Table 5.2.4.c) only seven of the nine materials had the same determination for testability by all labs in each study. Six of the materials were testable by all six labs and one material was determined to be not testable by all 6 laboratories. The CV's for between-laboratory reproducibility ranged from 3.9% to 57.8%.

The between laboratory reproducibility of the CM for materials common to the EC/HO study and the COLIPA study were also analyzed with respect to the predicted hazard classifications. These prediction models utilized to make these predictions are discussed in sections 5.2.2.3 and 5.2.3.3 for the EC/HO study and the COLIPA study, respectively.

Table 5.2.4.d illustrates the reproducibility for the surfactant materials, and Table 5.2.4.e illustrates the reproducibility of the non-surfactant materials. These results are summarized in Table 5.2.4.f for the surfactant materials and in Table 5.2.4.g for the non-surfactant materials.

The surfactant summary (Table 5.2.4.d) shows that when all 6 labs tested the material, the labs were in complete agreement for 5 out of 7 (71.4%) materials. For one material only 5 labs agreed and for 2 materials only 4 labs agreed. There was only one case where 5 labs tested the material, and in this case all 5 labs agreed.

For the non-surfactant materials agreement was not as good. Table 5.2.4.g shows that when all 6 labs tested the material, there was complete agreement for only 1 of 5 (20%) of the materials. Five labs agreed on three materials and four labs agreed on 2 materials. In the one case where only 5 labs tested the material, all five labs agreed on the hazard prediction.

Table 5.2.4.b Surfactant materials - Between laboratories reproducibility for 11 test materials in common for the EC/HO (Labs 30-33) and COLIPA (Company # 4 and Company # 5) studies. Company # 4 (Lab 27) and SM31 are the same laboratory. N = 11 surfactant materials. Annexes H13, F13 & H29.

Substance	COLIPA MRD_{50} (mg/mL)			EC/HO MRD_{50} (mg/mL)					Between Study Mean	Between Study
	Company # 4 (27)	Company # 5 (28)	Mean	SM 30	SM 31	SM 32	SM 33	Mean	MRD_{50} (mg/mL)	CV (%)
Benzalkonium chloride 1%	4.11	4.33	4.22	4.71	5.16	4.65	3.58	4.53	4.37	4.9
Benzalkonium chloride 10%	0.32	0.31	0.32	0.26	0.47	0.38	0.44	0.39	0.35	14.3
Benzalkonium chloride 5%	0.81	1.38	1.1	1.15	1.09	0.98	1.28	1.13	1.11	1.9
Cetylpyridinium bromide 10%	*	*	*	0.78	1.02	2.34	0.89	1.26	1.26	*
Cetylpyridinium bromide 6%	1.36	*	1.36	0.6	1.35	0.44	1.11	0.87	1.12	30.7
Polythylene glycol 400	296.50	316.23	306.37				363.92	363.92	335.14	12.1
Sodium lauryl sulphate 15%	0.52	0.51	0.51	0.62	0.6	0.51	0.74	0.62	0.57	12.9
Sodium lauryl sulphate 3%	3.23	2.78	3.01	2.71	3.04	3.74	3.64	3.28	3.14	6.2
Triton X-100 10%	2.47	1.24	1.86	1.61	1.96	1.5	2.22	1.82	1.84	1.2
Triton X-100 5%	4.66	2.42	3.54	1.9	3.39	5.09	2.53	3.23	3.38	6.5
Tween 20	9.50	3.49	6.5	1.52	5.53	4.98	1.06	3.27	4.88	46.6
Mean										**13.8**
Median										**9.3**

Table 5.2.4.c Non-surfactant materials - Between laboratories reproducibility for 9 test materials in common for the EC/HO (Labs 30-33) and COLIPA (Company # 4 and Company # 5) studies. Company # 4 (Lab 27) and SM31 are the same laboratory. N = 9 non-surfactant materials. Annexes H13, F13 & H29.

Substance	COLIPA MRD_{50} (mg/mL)			EC/HO MRD_{50} (mg/mL)					Between Study Mean	Between Study
	Company # 4 (27)	Company # 5 (28)	Mean	SM 30	SM 31	SM 32	SM 33	Mean	MRD_{50} (mg/mL)	CV (%)
Ethyl acetate	*	*	*	*	53.7	*	*	53.7	53.7	*
Glycerol	214.80	208.7	211.75	121.62	180.72	8.26	208.93	129.88	170.82	33.9
Imidazole	18.84	26.03	22.4	22.75	23.07	0.18	48.75	23.69	23.04	3.9
Isopropanol	52.60	124.5	88.55	83.18	91.2	87.1	143.55	101.26	94.9	9.5
Methyl ethyl ketone	54.20	*	54.2	55.72	50.47	78.16	47.97	58.08	56.14	4.9
n-Butyl acetate	*	*	*	*	*	*	*	*	*	*
Sodium hydroxide 1%	9.09	13.6	11.35	28.18	16.22	32.36	31.62	27.1	19.19	57.8
Sodium hydroxide 10%	4.33	0.6	2.47	2.28	1.6	2.67	2.49	2.26	2.36	6.2

Trichloroacetic acid 30%	1.12	1.24	1.18	1.69	2.47	0.81	2.2	1.79	1.49	29.2
Mean										**20.8**
Median										**9.5**

* - Participating laboratory did not test the chemical because it determined that chemical was not compatible with the test system.

Table 5.2.4.d Surfactant Materials - Between-laboratories reproducibility of Cytosensor Microphysiometer hazard classifications for test materials in common for the EC/HO (Labs 30-33) and COLIPA (Company # 4) studies. Company # 4 (Lab 27) and SM31 are the same laboratory. Cut-off values from Figures 6.1.3.4.1.b, 6.1.3.4.1.c, 6.1.3.4.2.b, 6.1.3.4.2.c, 6.1.3.4.3.b, and 6.1.3.4.3.c were applied. N = 11 surfactant materials. Annexes H13, F13 & H29.

Substance	EU						GHS						EPA					
	COLIPA		EC/HO				COLIPA		EC/HO				COLIPA		EC/HO			
	Company # 4 (27)	Company # 5 (28)	SM 30	SM 31	SM 32	SM 33	Company # 4 (27)	Company # 5 (28)	SM 30	SM 31	SM 32	SM 33	Company # 4 (27)	Company # 5 (28)	SM 30	SM 31	SM 32	SM 33
Benzalkonium chloride 1%	R36	R36	R36	R36	R36	R36	2A or 2B	2A or 2B	2A or 2B	2A or 2B	2A or 2B	2A or 2B	II or III	II or III	II or III	II or III	II or III	II or III
Benzalkonium chloride 10%	R41	R41	R41	R41	R41	R41	1	1	1	1	1	1	I	I	I	I	I	I
Benzalkonium chloride 5%	R41	R41	R41	R41	R41	R41	1	1	1	1	1	1	I	I	I	I	I	I
Cetylpyridinium bromide 10%	*	*	R41	R41	R36	R41	*	*	1	1	2A or 2B	1	*	*	I	I	II or III	I
Cetylpyridinium bromide 6%	R41	*	R41	R41	R41	R41	1	*	1	1	1	1	I	*	I	I	I	I
Polyethylene glycol 400	NL	NL	*	*	*	NL	NL	NL	*	*	*	NL	IV	IV	*	*	*	IV
Sodium lauryl sulphate 15%	R41	R41	R41	R41	R41	R41	1	1	1	1	1	1	I	I	I	I	I	I
Sodium lauryl sulphate 3%	R36	R36	R36	R36	R36	R36	2A or 2B	2A or 2B	2A or 2B	2A or 2B	2A or 2B	2A or 2B	II or III	II or III	II or III	II or III	II or III	II or III
Triton X-100 10%	R36	R41	R41	R41	R41	R36	2A or 2B	1	1	1	1	2A or 2B	II or III	I	I	I	I	II or III

Triton X-100 5%	R36	R36	R41	R36	R36	R36	2A or 2B	2A or 2B	1	2A or 2B	2A or 2B	2A or 2B	II or III	II or III	I	II or III	II or III	II or III
Tween 20	R36	R36	R41	R36	R36	R41	2A or 2B	2A or 2B	1	2A or 2B	2A or 2B	1	II or III	II or III	I	II or III	II or III	I

Table 5.2.4.e Non-Surfactant Materials - Between-laboratories reproducibility of Cytosensor Microphysiometer hazard classifications for test materials in common for the EC/HO (Labs 30-33) and COLIPA (Company # 4 and Company # 5) studies. Company # 4 (Lab 27) and SM31 are the same laboratory. Cut-off values from Figures 6.1.3.4.a, 6.1.3.4.b, and 6.1.3.4.c were applied. N = 9 non-surfactant materials. Annexes H13, F13 & H29.

Substance	EU						GHS						EPA					
	COLIPA		EC/HO				COLIPA		EC/HO				COLIPA		EC/HO			
	Company # 4 (27)	Company # 5 (28)	SM 30	SM 31	SM 32	SM 33	Company # 4 (27)	Company # 5 (28)	SM 30	SM 31	SM 32	SM 33	Company # 4 (27)	Company # 5 (28)	SM 30	SM 31	SM 32	SM 33
Ethyl acetate	*	*	*	R36	*	*	*	*	*	2A or 2B	*	*	*	*	*	III	*	*
Glycerol	NL	NL	NL	NL	R36	NL	NL	NL	NL	NL	2A or 2B	NL	IV	IV	IV	IV	II or III	IV
Imidazole	R36	R36	R36	R36	R41	R36	2A or 2B	2A or 2B	2A or 2B	2A or 2B	1	2A or 2B	II or III	II or III	II or III	II or III	I	II or III
Isopropanol	R36	NL	NL	NL	NL	NL	2A or 2B	NL	NL	NL	NL	NL	II or III	IV	IV	IV	IV	IV
Methyl ethyl ketone	R36	*	R36	R36	R36	R36	2A or 2B	*	2A or 2B	2A or 2B	2A or 2B	2A or 2B	II or III	*	II or III	II or III	II or III	II or III
n-Butyl acetate	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Sodium hydroxide 1%	R36	R36	R36	R36	R36	R36	2A or 2B	2A or 2B	2A or 2B	2A or 2B	2A or 2B	2A or 2B	II or III	II or III	II or III	II or III	II or III	II or III

Sodium hydroxide 10%	R36	R41	R41	R41	R41	R41	2A or 2B	1	1	1	1	1	II or III	I	I	I	I	I
Trichloroacetic acid 30%	R41	R41	R41	R41	R41	R41	1	1	1	1	1	1	I	I	I	I	I	I

Table 5.2.4.f Surfactant Materials - Between-laboratories reproducibility of Cytosensor Microphysiometer hazard classifications agreement for test materials in common for the EC/HO (Labs 30-33) and COLIPA (Company # 4 and Company # 5) studies. Company # 4 (Lab 27) and SM31 are the same laboratory.

Where all 6 labs tested the material			
Agreement	EU	GHS	EPA
6 labs	5	5	5
5 labs	1	1	1
4 labs	2	2	2
3 labs	0	0	0
<3 labs	0	0	0
Where 5 labs tested the material			
Agreement	EU	GHS	EPA
5 labs	1	1	1
4 labs	0	0	0
3 labs	0	0	0
<3 labs	0	0	0
Where 4 labs tested the material			
Agreement	EU	GHS	EPA
4 labs	0	0	0
3 labs	1	1	1
<3 labs	0	0	0

Table 5.2.4.g Non-Surfactant Materials - Between-laboratories reproducibility of Cytosensor Microphysiometer hazard classifications agreement for test materials in common for the EC/HO (Labs 30-33) and COLIPA (Company # 4 and Company # 5) studies. Company # 4 (Lab 27) and SM31 are the same laboratory.

Where all 6 labs tested the material			
Agreement	EU	GHS	EPA
6 labs	2	2	2
5 labs	4	4	4
4 labs	0	0	0
3 labs	0	0	0
<3 labs	0	0	0
Where 5 labs tested the material			
Agreement	EU	GHS	EPA
5 labs	1	1	1
4 labs	0	0	0
3 labs	0	0	0
<3 labs	0	0	0

5.2.5 Analysis of materials common to both the CTFA and COLIPA studies

Since there were also surfactant-based formulations which were common to both the CTFA and COLIPA studies it seemed reasonable to use these materials to help assess the between laboratory reproducibility of the CM assay. This seemed as if it would be especially useful since one lab (Company # 4) participated in both studies. However, it is known that there were several differences in the studies, one of which is that the common formulations had to be remade for the COLIPA study using the same formulas as the CTFA study. This is problematic because it is possible, if not likely, that due to the several years difference between the studies that the composition and/or purity of many of the ingredients would be different.

To determine if this was the case we first looked at the rabbit scores for the 6 materials that were common to the two studies. Table 5.2.5 shows that while three of the materials (Baby shampoo 2, Eye Makeup Remover, and Skin Cleanser) had identical hazard classifications between the two studies, the other three materials (Liquid Soap 1, Shampoo 1, and Gel Cleanser) had widely disparate scores. Considering just the EU classifications, Liquid Soap 1 and Shampoo 1 both went from a CTFA study Not Classified to a COLIPA study R41. The Gel Cleanser hazard categories changed in the opposite direction – from an R41 in the CTFA study to a Not Classified in the COLIPA study.

Thus we concluded that it was likely that the composition of some of the materials differed between the studies (although the Draize test also differed since local anesthesia was used in the CTFA study but not in the COLIPA study), and therefore it would not make sense to attempt to estimate reproducibility of the *in vitro* test using data of such uncertainty.

Table 5.2.5 Between-laboratories reproducibility of Cytosensor Microphysiometer hazard classifications for test materials in common for the CTFA and COLIPA studies. Hazard classifications are based on *in vivo* Draize data.

	CTFA						COLIPA					
Substance Designation	Conc. Tested	n. of animals	EU	GHS	EPA	CM converted value MRD_{50} (mg/mL)	Conc. Tested	n. of animals	EU	GHS	EPA	Average MRD_{50} (mg/mL) (avg MA & CT)
Liquid Soap 1	25%	6	Not classified	No category	Category III	2.80	100%	3	R41	Category 1	Category I	0.78
Gel Cleanser*	100%	6	R41	No category	Category I	3.19	100%	3	Not classified	No Category	Category III	5.58
Baby Shampoo 2	100%	6	R41	Category 1	Category I	1.50	100%	3	R41	Category 1	Category I	2.33
Shampoo 1*	25%	6	Not classified	No category	Category III	1.72	100%	3	R41	Category 1	Category I	0.735
Eye Makeup Remover	100%	6	Not classified	No category	Category IV	20.0	100%	3	Not classified	No Category	Category IV	93.5
Skin Cleaner*	100%	6	R41	Category 1	Category I	1.09	100%	3	R41	Category 1	Category I	0.696

* - COLIPA formulation slightly different than the CTFA formulation

5.3 Compilation of results, statistical approaches used: description & rationale for the approach used to determine between-laboratory reproducibility

Reproducibility was assessed in the forgoing sections mainly by calculating mean MRD_{50} values from individual CM or SM runs for a specific test material and comparing these values to the mean MRD_{50} values for the same material when conducted in another laboratory or in several other laboratories. The mean MRD_{50} values from the individual laboratories were compared by calculating their mean and SD and then calculating the CV. We feel that this is a reasonable method of comparison when the results from at least three laboratories are being compared, but in some cases data from only two laboratories are available, e.g. the COLIPA study. Because of the small sample size on which it is based, we feel that in these cases the CV is probably not an exceptionally strong statistic.

In some cases it was possible to present correlation coefficients to compare groups of laboratories. These statistics were valuable in such instances as the EC/HO study where 4 laboratories were involved. By conducting a lab vs. lab comparison, we were able to determine if one of the groups of laboratories appeared to be an "outlier" from the others. However, correlation coefficients do not address how closely actual values match each other, only the trends between a set of values. Two laboratories which have good correlation coefficients could actually have dissimilar values as long as one lab was always consistently higher or lower than the other lab.

We also investigated whether the hazard predictions produced by the participating laboratories were reproducible. We presented this information in a detailed tabular form as well as in summary tables. The only statistic that we used was the percentage of materials for which all (or a stated portion) of the laboratories predicted identical hazard classifications. We were unable to find a more quantitative measure of reproducibility for the hazard classifications.

5.4 Additional studies where raw data are not available: attempt to combine the data using weight-of-evidence approaches

The two major studies (COLIPA and EC/HO) (plus an analysis of the overlapping test materials between the studies) and the one smaller study (Bagley, Bruner et al. 1992) reported here give the best indication of the between laboratory reproducibility of the assay. These studies were performed with blinded test materials, in multiple laboratories, in different countries, and with different sources of media. However, with one exception, the results appeared to be quite comparable across a range of materials with the caveat that only materials that are water–soluble can be adequately tested. These were the only studies where identical test materials were tested in at least two different laboratories. We are unaware of other studies which have overlapping materials that could be used to combine with these studies in a weight of evidence approach.

6. Predictive Capacity (Module 5)

As discussed in Module 1, the CM assay monitors the real time action of increasing test article dilutions on a monolayer culture of cells. The cytotoxicity of the L929 cells is observed by the CM as a decrease in the pH surrounding the cells. It has been proposed that similar cytotoxic actions of test materials on the epithelium, stroma, and endothelial cells of the human eye are a major factor in causing ocular irritation (Jester, Petroll et al. 1998; Jester, Li et al. 2001; Maurer, Parker et al. 2002). Historically, the materials tested in the CM assay have been surfactant-based household cleaning and personal care products and ingredients. Because of the restrictions of this dilution based assay, it is currently used primarily for evaluating the eye irritation potential of liquid surfactant containing formulations and mixtures.

Although data from human experience (e.g. accidental exposure) or approved clinical studies would be the best way to assess the predictive capacity of the CM, almost all studies cited in this BRD have used the Draize rabbit eye test MAS or MMAS values as the standard for a quantitative measure of eye irritation. One exception was the CTFA Phase III study which also evaluated the CM by its ability to predict the US Federal Hazardous Substance Act categories or the Kay-Calandra hazard categories. None of the studies addressed the ability to address the EU, GHS or EPA hazard classifications as we have in this BRD.

Regardless of the fact that summarized Draize MAS or MMAS scores were routinely used in the cited manuscripts, we were able to obtain raw data from the animal tests of approximately half of the studies evaluated here. This allowed us to calculate the EU, GHS or EPA irritation categories based on published criteria. For this subset of studies we then used the EU, GHS, and EPA categories as the standard against which we could judge the predictive capacity of the CM test. For studies where only summary data were available we report the predictive capacity based on the ability to predict Draize MAS or MMAS scores.

A significant problem in analyzing how well any *in vitro* test predicts the outcome of an *in vivo* test is that a single value is generally associated with the animal score and this single value is treated as a "gold standard". In reality, there is no single eye irritation value that characterizes a test material; the value that is obtained will generally vary each time the material is tested. Thus it is extremely unlikely that an *in vitro* score and an *in vivo* score will match exactly, no matter how perfect the *in vitro* test is performed. This fact is often overlooked in most validation studies. Generally the animal score is treated as a single fixed value (since the animal test is generally conducted only once), and the *in vitro* test is then assessed for its "accuracy" based on how well its data match that of the animal test. Only a few studies, e.g the CTFA Phase III study, have taken the animal test variability into account. The CTFA study used bootstrap resampling to estimate with-in group variability for each test material so that Draize scores could be represented more realistically with their variability.

As mentioned above, one reason that Draize MAS scores are usually treated as unvarying values is that both ethical and financial considerations generally demand that a rabbit eye test only be conducted a single time. Thus for many materials there is no information about what score might occur in a repeat test, and without the results of multiple tests it is difficult to address variability.

However, there is one approach which can supply some quantitative insight into this problem. Because over the years the Draize test protocol has evolved from a six rabbit test to a three rabbit test, there is one way of estimating variability for materials which were tested with the six rabbit protocol. It is possible to analyze the ocular response of the six rabbits by placing them into smaller groups. For example, the results for each of the six individual rabbits can be recombined into multiple unique groups of three rabbits (matching the number of rabbits used in today's standard protocol). In fact, all rabbits (designated A – F in the following example) in a six rabbit test can be recombined into 20 unique three rabbit groups, e.g. ABC, ABD, ABE, ABF, etc. This is an approach already used by others in studies to determine the necessary sample size for a rabbit ocular irritation test (DeSousa, Rouse et al. 1984). Each three rabbit group can then be given a hazard classification according to the published guidelines from specific regulatory bodies. The number of subgroups in each hazard classification can then be viewed as a measure of the variability of the test. If all 20 subgroups are classified as R36, for example, then the R36 classification for that material can be considered not very variable. However, if 10 subgroups are rated as No Label and the other 10 are rated as R41, then the results for that material would be considered quite variable. In essence the above results mean that if the material were tested in multiple three rabbit tests, half of the tests would rate it as a very severe R41 material, and the other half of the tests would rate it as a mild No Label material. Therefore, an *in vitro* test of the same material should not necessarily be expected to always make a prediction of R41, which would be the overall prediction of the six rabbit test.

To demonstrate the level of Draize test variability which occurs in the real world, we have examined the animal data from the CTFA Phase III study. This study had arguably one of the best controlled animal studies because it was conducted under GLP's and utilized a randomized block design (3 males and 3 females) with each animal's dosing initiated on a separate day.

Table 6.0 shows for the CTFA Phase III study the number of three rabbit subgroups which fall into each of the hazard categories for the three regulatory classification schemes (GHS, EU, and EPA). Data which support these classifications can be found in spreadsheets contained in Annex I) CTFA Animal Data) It can be seen that in some cases all of the three rabbit subgroups give the same hazard classification as the six rabbit study, e.g. the EU classification for HZB, HZC and HZD is No Label, and each of the 20 three rabbit subgroups for each test material is also No Label. However, for those same three test materials classified by GHS criteria there is considerable difference between the subgroups and the original six rabbit study. For example, HZC is No Label by the six rabbit test, but only half (10) of the three rabbit groups are No Label; seven are 2B and 3 are category 1. This means if the test were repeated 20 times using the current three rabbit

protocol there would be an equal chance of having a higher than No Label score (10 out of 20 times) as there would be of having the No Label score (10 out of 20 times). Similar results can be seen for many of the materials in this study.

Even more dramatic examples can be found in the CTFA Phase III study. HZE, for example, is classified R41 by the six rabbit test, but only 10 of the subgroups have R41 classifications, the other 10 are No Label! Thus if the three rabbit test were run only once, there would be a 50% chance of having the lowest classification (No Label) and an equal chance of having the highest label (R41). HZP is another interesting example. Although it has a 6-rabbit GHS classification of No Label, 6 out of 20 tests (30% of the time) give a Category 1 result – three categories higher than that determined by the 6 rabbit test! Other interesting examples are highlighted in bold in the table.

Table 6.0 Recombination of each 6 rabbit test result into 20 three rabbit test subgroups. Each subgroup was classified separately according to the rules for each of the three classification systems, and the number of subgroups falling into each hazard category is indicated. Numbers in bold, shaded areas represent results from test materials where the subgroups differed in their hazard classification from the overall six rabbit classification. Data from the CTFA Phase III study. N = 25 materials. Raw animal data from Annexes I55 & I3.

		6 animal study score			GHS Counts				EU Counts			EPA Counts			
		GHS	EU	EPA	1	2A	2B	NL	R41	R36	NL	I	II	III	IV
Shampoo 7	HZA	1	R41	1	**16**	**4**	0	0	**16**	**3**	**1**	**16**	**4**	0	0
Liquid Soap 1	HZB*	NL	NL	3	0	0	**4**	**16**	0	0	20	0	0	20	0
Shampoo 1	HZC*	NL	NL	3	**0**	**0**	**10**	**10**	0	0	20	0	0	20	0
Shampoo 5	HZD*	NL	NL	3	0	0	0	20	0	0	20	0	0	20	0
Gel Cleaner	HZE	NL	R41	1	**10**	**0**	**0**	**10**	**10**	**0**	**10**	**10**	**0**	**10**	0
Baby Shampoo 2	HZF	1	R41	1	**16**	**4**	0	0	**16**	**3**	**1**	**16**	**4**	0	0
Shampoo 8	HZG*	NL	NL	3	**0**	**0**	**0**	**20**	0	0	20	0	0	20	0
Eye Makeup re.	HZH	NL	NL	4	0	0	0	20	0	0	20	0	0	0	20
Skin Cleaner	HZI	1	R41	1	**19**	**1**	0	0	**19**	**1**	0	**19**	**1**	0	0
Mild Shampoo	HZJ	NL	NL	4	0	0	0	20	0	0	20	0	0	0	20
Bubble bath	HZK	1	R41	1	20	0	0	0	20	0	0	20	0	0	0
Foam Bath	HZL	1	R41	1	**19**	**0**	**1**	0	**19**	**0**	**1**	**19**	**0**	**1**	0
Shampoo 3	HZM*	NL	NL	3	0	0	0	20	0	0	20	**0**	**0**	**10**	**10**
Shampoo 6	HZN*	NL	NL	3	0	0	0	20	0	0	20	0	0	20	0
Baby Shampoo 1	HZP	NL	NL	3	**0**	**0**	**0**	**20**	0	0	20	0	0	**19**	**1**
Cleaning Gel	HZQ	NL	NL	3	**0**	**0**	**0**	**20**	0	0	20	0	0	20	0
Facial Cleaning Foar	HZR*	NL	R41	1	**10**	**0**	**3**	**7**	**10**	**0**	**10**	**10**	**0**	**10**	0
Shower Gel	HZS	1	R41	1	**19**	**1**	**0**	0	**19**	**1**	0	**19**	**1**	0	0
Polishing Scrub	HZT	NL	NL	4	0	0	0	20	0	0	20	0	0	0	20
Hand Soap	HZU*	NL	NL	3	**0**	**0**	**4**	**16**	0	0	20	0	0	20	0
Shampoo 4	HZV*	NL	NL	3	0	0	0	20	0	0	20	0	0	20	0
Liquid Soap 2	HZW*	2B	NL	3	**0**	**0**	**16**	**4**	0	0	20	0	0	20	0
Shampoo 2	HZX	1	R41	1	**19**	**1**	0	0	**19**	**0**	**1**	**16**	**4**	0	0
Shampoo AntiD	HZY	1	R41	1	**16**	**4**	0	0	**16**	**4**	0	**16**	**4**	0	0
Facial Cleaner	HZZ	NL	NL	4	0	0	0	20	0	0	20	0	0	0	20

* tested at 25% (w/v) in vivo and in vitro (starting material)

The conclusion from studying this example is that neither a Draize MAS score nor a hazard classification is an unvarying physical constant for the test material. Therefore, an *in vitro* test should not be expected to exactly match a hazard category determined *in vivo*

because the next time the animal test is run it might also fail to match the hazard classification of the first animal test.

6.1 Studies with available raw data

There were 4 studies where sufficient raw data was available to attempt to determine predictive capacity for regulatory classification schemes. For the vast majority of the test materials in the EC/HO study, the CTFA Phase III study, and the COLIPA study, individual animal and tissue data from a traditional Draize test were available and sufficient to allow the unequivocal determination of the EU, GHS, or EPA eye irritation category. However, some of the animal tests were conducted in such a way that all the appropriate data for the determination of EU, GHS, or EPA category were not available. In these cases, the test material was left out of the analysis.

It should also be noted that the Draize test for the CTFA Phase III study was conducted using ocular anesthesia, whereas the Draize tests for the EC/HO study and the COLIPA study did not use anesthesia. It is expected that the eye irritation results for the same compounds may differ because of this difference in protocol.

For the Company # 1 unpublished data studies, the animal ocular irritation tests were conducted using the Low Volume Eye Test (LVET). The LVET uses the same scoring scale as the Draize test, but is conducted with one-tenth the volume (100 µL) applied to the center of the cornea as opposed to the conjunctival sac. The LVET was used since it has been proposed to be more predictive (yet still over predictive) of the human response than the traditional Draize test (Griffith, Nixon et al. 1980; Freeberg, Nixon et al. 1986).

Method of analysis

In general, all of the following analyses were conducted in a similar fashion. First the raw animal data, which consisted of tissue scores for individual animals taken at designated time points until the lesions had cleared or until 21 days had elapsed, were inserted into spreadsheets constructed to apply the rules established by the GHS, EU, and EPA scoring systems. These spreadsheets then returned the classification for that material. In some cases, the appropriate data from the animal test had not been recorded or supplied for the chemical or formulation in question. In cases where the hazard classification of the test material could not be unambiguously categorized, it was dropped from the analysis. Thus some of the graphs in the following sections will have fewer data points than the number of test materials listed in some of the previous tables.

Next, the assigned categories were given a scale ranking from 1 (highest) to 3 (lowest for the EU scale) or 4 (lowest for the GHS and EPA scale) and then matched with the appropriate *in vitro* score. These paired numbers were then graphed (GraphPad Prism®, GraphPad Software, Inc., San Diego, CA) on an XY graph with the abscissa being the numerical hazard category. Additional axis labels were then added to indicate the hazard class, and at the same time the now duplicative numerical rankings were removed.

Specific breakpoints between hazard categories could then be visualized as places where *in vitro* cut-off lines could be drawn.

These cut-off values, i.e. prediction models, determined post-hoc were sometimes slightly different from the proposed prediction models which had been submitted to the ECVAM management team in September 2006. The changes occurred because the originally proposed cut-offs were based on only preliminary analysis of hazard category data. Prior to this time the only published PM for the CM had been for the prediction of Draize scores. See Section 2.2.5 of this BRD for a more detailed description of the determination of the prediction models.

For studies where insufficient animal data were supplied to determine the hazard classification, an XY graph was constructed relating the *in vitro* scores to the Draize score. Although these graphs will not be directly helpful in determining the predictivity of the SM or CM for hazard classes, they may be helpful in assessing the general ability of the *in vitro* test to identify more or less irritating materials.

Subsequent to the construction of the scatter plots with the proposed cut-offs for the various hazard classifications, we prepared contingency tables to summarize the performance of the CM test and its prediction model in each of the major studies (and subsets of the studies where specific chemical classes were investigated). The parameters used to summarize the performance are reasonably standard for the analysis of toxicity tests and are defined below:

Concordance – the percentage of materials predicted by the CM to have the same hazard classification as determined by the rabbit test

Predictivity – the proportion of materials assigned to a specific hazard category by the animal test which were assigned to the same category by the CM.

In addition, analyses of how well the CM performed in separating "severe irritants" (those materials in the highest irritation category of any of the classification systems) from the rest (those in the remaining categories). The parameters used in this analysis are:

Concordance – the percentage of correctly identified severe irritants and those materials which were not severe irritants (a combination of mild irritants and non-irritants).

Sensitivity – the number of correctly identified severe irritants by the CM assay as a proportion of the number of actual severe irritants.

Specificity – the number of correctly identified non-severe irritants ("the rest") as a proportion of the total number of actual non-severe irritants.

Positive Predictivity –the number of correctly predicted severe irritants as a proportion of the total number of predicted severe irritants.

Negative Predictivity –the number of correctly predicted non-severe irritants ("the rest") as a proportion of the total number of predicted non-severe irritants.

False Positive Rate – the number of non-severe irritants predicted by the CM to be severe irritants as a proportion of the total number of non-severe irritants.

False Negative Rate – the number of severe irritants predicted to be non-severe irritants as a proportion of the total number of severe irritants.

A third analysis of the ability of the CM to separate non-irritants (those materials in the lowest irritation category of any of the classification systems) from the rest (those in the remaining categories). The parameters used in this analysis are:

Concordance – the percentage of correctly identified non-irritants and irritants.

Sensitivity – the number of correctly identified irritants (e.g R36 plus R41) by the CM assay as a proportion of the number of actual irritants.

Specificity – the number of correctly identified non-irritants by the CM assay as a proportion of the total number of actual non-irritants.

Positive Predictivity – the number of correctly predicted irritants as a proportion of the total number of predicted irritants i.e. this analysis only determined that irritants were predicted to be irritants by the CM assay but did not distinguish between the different irritancy classifications predicted (e.g., R36 and R41).

Negative Predictivity – the number of correctly predicted non-irritants as a proportion of the total number of predicted non-irritants.

False Positive Rate – the number of non-irritants predicted to be irritants as a proportion of the total number of non-irritants.

False Negative Rate – the number of irritants predicted to be non-irritants as a proportion of the total number of irritants.

6.1.1 Relevant information for each study where raw data are available

Table 6.1.1 Table presenting the relevant information for each study where raw data are available

Studies	No. of chemicals	No. of products	Coded	No. of labs	No. of runs/ exp	Data format (raw, summary)	Chemical classes covered	Ranges of toxicity covered	Physico-chemical properties covered
EC/HO[1] (Balls, Botham et al. 1995)	31 of 60 tested	0	Coded	4	3-4	Raw data	Alcohols, surfactants, ketones, bases, acids, esters	MAS 0-108 EU: R36, R41, NC GHS: 1, 2A, 2B, NC EPA: I-IV	See Annex A and B
CTFA[2] (Gettings, Lordo et al. 1996)	0	24 of 25 tested	Coded	1	3	Raw data	Personal care products and their ingredients, mostly surfactant based	MAS 2.3-44 EU: R41, NC GHS: I, 2A, 2B, NC EPA: I-IV	Liquids and water-soluble materials
COLIPA[1] (Brantom, Bruner et al. 1997; Harbell, Osborne et al. 1999)	17 of 23 tested	9 of 32 tested	Coded	2	3	Raw data	Alcohols, surfactants, ketones, bases, acids, esters	MAS 0–106 EU: R36, R41, NC GHS: 1, 2A, 2B, NC EPA: I-IV	See Annex A and B
Company 1[1 & 2] (Unpublished)	76 unique of 101 tested		Coded	1	≥3	Raw data	Household and personal care products and ingredients	LVET MAS 0-65 EU: R36, R41, NC GHS: 1, 2A, 2B, NC EPA: I-IV	Liquids and water-soluble materials

[1] Performed using the Cytosensor transwell protocol with an 810-second exposure and L929 cells.
[2] Performed using the silicon microphysiometer coverslip protocol with a 500-second exposure and L929 cells.

6.1.2 *In vivo* reference data used to assess the performance of the alternative method

Most of the reference data for the larger validation studies were collected from the exposure of rabbits using the standard Draize methodology. Within this general protocol; however, there could be significant variations including the use of anesthesia, the use of 1 to 12 animals, level of GLP compliance, level of blinding of the study, etc. In addition, most of the studies suffered from the confounder that historical data was used for the reference. In such studies it is often difficult, if not impossible, to determine after the fact (perhaps many years later) the composition and purity of the materials that were originally tested. Thus it becomes problematic whether the materials tested *in vitro* are at all similar to the materials that were tested *in vivo*.

Of the studies analyzed in this BRD, only the CTFA Phase III study utilized a concurrent testing scheme for all the test materials (Brantom, Bruner et al. 1997). Single large lots of the formulations to be tested were divided between the animal studies and the *in vitro* studies. Thus there was confidence that the materials used for *in vitro* testing were indeed the same materials that were tested *in vivo*. Although all of the *in vivo* data were collected before the *in vitro* testing began, the time differential was so small that it is very unlikely that significant changes occurred in the formulations before the *in vitro* testing commenced. COLIPA used concurrent testing for the 32 formulations which were formulated specifically for their program (Brantom, Bruner et al. 1997) following formulations used for the CTFA Phase I (Feder, Lordo et al. 1991), Phase II (Gettings, Dipasquale et al. 1994), and Phase III (Gettings, Lordo et al. 1996) evaluations.

Although the larger validation studies utilized a more or less standard Draize test, the extensive internal data supplied by the Company # 1 used the LVET as the reference standard. The LVET is similar to the Draize test (e.g. it has identical tissue scoring), but it uses a smaller dose of test material. The volume of material applied to the eye in the LVET is 10 µl – ten times less than the 100 µl applied in the traditional Draize. In addition, the LVET protocol calls for the test material to be applied directly to the surface of the cornea, while in the traditional Draize the test material is placed inside the conjunctival sac. The rational behind the LVET protocol is that it is said to be more predictive of the human reaction to an accidental ocular exposure than the Draize test which is thought to significantly over predict the human reaction. Support for this view comes from studies which show that when controlled human clinical studies are conducted concurrently with both the traditional Draize and the LVET, the LVET more closely matches the clinical response (Freeberg, Nixon et al. 1986). In general, both protocols seem to over predict the human response, but the LVET is claimed to over predict by a smaller amount than the traditional Draize. When LVET data are used as part of determining the predictive capacity of the CM or SM, that fact will be clearly noted. Table 6.1.2 provides a general description of the study design and methods of collection for the animal reference data examined in this BRD.

Table 6.1.2 Table presenting the *in vivo* reference data available

Studies	Species and protocols used as reference data	Sources of information	n. of labs	n. of chem	n. of animals	Quality of the reference data	Format of data available
EC/HO[1] (Balls, Botham et al. 1995)	Albino rabbits using traditional Draize and OECD TG405. No anesthesia. Whether every chemical was tested this way could not be determined	(Balls, Botham et al. 1995)	Labs not identified; number unknown	31 of 60 tested	>3	*In vivo* – principles of GLP *In vitro* – principles of GLP for at least 1 lab	*In vivo* – individual animal tissue scores *In vitro* – summaries (means); some raw data have been obtained (Annex L)
CTFA[2] (Gettings, Lordo et al. 1996)	Albino rabbits using traditional Draize and an experimental block design. Anesthesia used.	(Gettings, Lordo et al. 1996)	1	24 of 25 tested	6 total; 3 males, 3 females	*In vivo* GLP *In vitro* – no Concurrent testing	*In vivo* – individual animal tissue scores *In vitro* – each trial Raw data in Annex I
COLIPA [1] (Brantom, Bruner et al. 1997)	Assumed to be albino rabbit using traditional Draize test, but protocols varied for the formulations. No anesthesia used	(Brantom, Bruner et al. 1997)	Labs not identified; number unknown for 23 materials; 1 lab for 32 formulations	26 of 55 tested	Varied, 1-6	*In vivo* – GLP status not known *In vitro* - no	*In vivo* – individual animal and tissue scores *In vitro* – each trial Raw data in Annex J
Company 1[1&2] (Unpublished)	Albino rabbit using LVET protocol. No anesthesia used.	Communication from the Company 1	Labs not identified; number unknown	76 of 101 tested	Varied, 1-6	Generally GLP	Complete animal data (LVET) and summary MRD_{50} Raw data in Annex K

[1] Performed using the Cytosensor transwell protocol with an 810-second exposure and L929 cells.
[2] Performed using the silicon microphysiometer coverslip protocol with a 500-second exposure and L929 cells.

6.1.3 Brief description of the studies with available raw data

6.1.3.1 Analysis of the EC/HO study (Balls, Botham et al. 1995)

The reference data for the EC/HO study are all from historic tests; consequently it is difficult to know whether lack of concordance between the animal study and the *in vitro* study for individual chemicals is due to the result of underperformance of the *in vitro* test or to an intrinsic difference between the chemical that was tested in the animal and the chemical that was tested *in vitro*. Positive points about the *in vivo* data used for this study are that the tests were supposed to have been conducted since 1981 and according to OECD TG405 following the principles of GLP. The tests were not to have used anesthesia and to have been conducted long enough to enable reversibility/irreversibility to be assessed. Although the above were stated to be general rules for the selection of data, it is not clear from the publication whether all of these conditions were actually met for each of the chemicals selected for the study. Only some raw data could be obtained and they are shown in Annex L.

Four laboratories contributed CM data for this study (Annex H13), but there was not unanimity as to which chemicals could be appropriately tested in the CM and which could not. Consequently there are not four MRD_{50} values for each chemical. Further analysis was carried out by only using data where two or more labs produced results. Therefore, unless noted, all the graphs in this chapter contain mean MRD_{50} data (from 2, 3, or 4 labs) for 31 chemicals. See section 5.2.2.2 for more detail.

An overall summary of the EC/HO study including the chemical identities, animal scores and *in vitro* scores are given in Table 6.1.3.1.a. Graphical presentations of the results, for all materials tested by 2, 3, or 4 labs are given in Figure 6.1.3.1.a for the EU classifications, Figure 6.1.3.1.b for the GHS classifications, and Figure 6.1.3.1.c for the EPA classifications.

Table 6.1.3.1.a Summary of the EC/HO study (Balls, Botham et al. 1995). Surfactant materials are highlighted. N = 20 non-surfactant materials and N = 11 surfactant materials.

In Vivo Eye Irritation Classifications - EC/HO study

EC/HO chemical number	Substance	CASRN[1]	Concentration Tested	Purity (%)	n. of animals	*In Vivo* EU[2,3]	*In Vivo* GHS[4,5]	*In Vivo* EPA[4,7]	ECETOC MMAS Score[8]	MRD_{50} (mg/mL) [11]
1	Sodium hydroxide (10%)	1310-73-2	10%	reagent grade	1	R41	Category 1	Category I	108	2.26
2	Benzalkonium chloride (10%)	63449-41-2	10%	98	3	R41	Category 1	Category I	108	0.39
3	Trichloroacetic acid (30%)	76-03-9	30%	reagent grade	1	R41	Category 1	Category I	106	1.79
4	Cetylpyridinium bromide (10%)	140-72-7	10%	99	6	R41	Category 1	Category I	89.7	1.26
5	Cetylpyridinium bromide (6%)	140-72-7	6%	99	4	R41	Category 1	SCNM[9]	85.8	0.87
6	Benzalkonium chloride (5%)	63449-41-2	5%	98	4	R41	Category 1	Category I	83.8	1.13
9	Cyclohexanol	108-93-0	100%	97	4	R41	Category 1	Category I	79.8	8.03
11	Promethazine HCl	58-33-3	100%	98	3	R41	Category 1	SCNM	71.7	1.27
13	Triton X-100 (10%)	9002-93-1	10%	98	6	R41	Category 1	Category II	68.7	1.82
14	Acetone	67-64-1	100%	99	4	R36	Category 2A	Category II	65.8	148.82
18	Isobutanol	78-83-1	100%	99.9	4	R36	Category 2A	Category II	60.3	27.91
19	Imidazole	288-32-4	100%	99	3	R41	Category 1	SCNM	59.3	23.69
20	Sodium lauryl sulfate (15 %)	151-21-3	15%	98	6	R41	Category 1	Category I	59.2	0.62
23	Methyl ethyl ketone	78-93-3	100%	99	4	R36	Category 2A	Category III	50	58.08
24	Pyridine	110-86-1	100%	>99.9	3	R41	Category 1	SCNM	48	19.73
26	Benzalkonium chloride (1 %) (1x)	63449-41-2	1%	98	4	R41	Category 2A	Category I	34.4	4.53
28	Gammabutyrolactone	96-48-0	100%	>99	3	R36	Category 2A	Category II	43	93.79
31	Methyl acetate	79-20-9	100%	98	4	R36	Category 2A	Category II	39.5	94.68
34	Triton X-100 (5 %) (1x)	9002-93-1	5%	98	6	R36	Category 2A	Category III	32.3	3.23
36	Isopropanol	67-63-0	100%	99.9	4	Not classified	Category 2A	Category III	30.5	101.26
37	Sodium perborate, $4H_2O$	10486-00-7	100%	98.6	6	R41	Category 1	Category I	30.5	1.69
39	2,5-Dimethylhexanediol	110-03-2	100%	99.5	3	R41	Category 1	Category I	28.3	98.67
40	Methyl cyanoacetate	105-34-0	100%	99	3	R36	Category 2A	Category II	27.7	21.54
41	Sodium hydroxide (1%)	1310-73-2	1%	reagent grade	4	R36	Category 2B	Category III	25.8	27.04
42	Ethanol	64-17-5	100%	n.p.	3	Not classified	Category 2A	Category III	24	111.99
44	Ammonium nitrate	6484-52-2	100%	99.999	3	R36	Category 2A	Category III	18.3	71.27
46	Sodium lauryl sulfate (3 %)	151-21-3	3%	98	6	Not classified	No category	Category III	16	3.28
53	Trichloroacetic acid (3%)	76-03-9	3%	reagent grade	6	Not classified	No category	Category III	6.7	15.03
55	Tween 20	9005-64-5	100%	98	4	Not classified	No category	Category III	4	3.27
58	Cetylpyridinium bromide (0.1%)	140-72-7	0.10%	99	6	Not classified	No category	Category III	2.7	84.65
59	Glycerol	56-81-5	100%	>99.5	6	Not classified	No category	Category IV	1.7	129.88

[1]CASRN=Chemical Abstract Services Registry Number

[2]EU=European Union (EU [2001]).

[3]Risk phrase R41 = risk of serious damage to the eyes; R36 = irritating to the eyes; not classified.

[4]GHS=Globally Harmonized System (UN [2003])

[5]Eye Irritant Category 1 = irreversible effects on the eye/serious damage to the eye; Category 2A = reversible effects on the eye/irritating to the eyes; Category 2B = reversible effects on the eye/mildly irritating to the eyes; No category

[6]EPA=U.S. Environmental Protection Agency (EPA [1996]).

[7]Toxicity Category I for the Primary Eye Irritation Study = Corrosive, or corneal involvement or irritation not reversible within 21 days; Category II = Corneal involvement or irritation clearing in 8-21 days; Category III = Corneal involvement or irritation clearing in 1-7 days; Category IV: minimal effects clearing in less than 24 hr

[8]MMAS scores reported in Balls et al. (1995) and in the ECETOC Technical Report n. 48 (1998)

[9]SCNM=Study Crtieria Not Met

[10]n.p.=not provided

[11] Mean of the 2, 3, or 4 labs reporting results

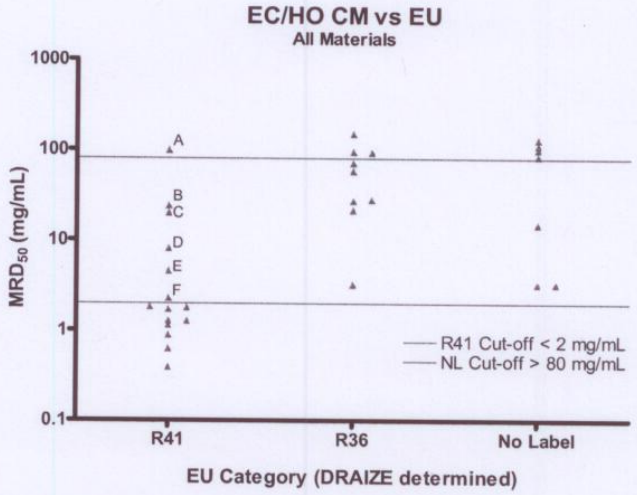


Figure 6.1.3.1.a Results of the EC/HO study related to EU classification. Data points indicate the mean MRD_{50} for the laboratories (2, 3, or 4) that provided data for that chemical. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude. All materials including surfactants are included.

Table 6.1.3.1.b Identification of the six R41 materials which were underpredicted by EU classification. The lettering system remains the same for the GHS and EPA scatterplots.

Graph Letter	Material	Chemical Class	Mean MRD_{50}
A	2,5-dimethylhexanediol	Alcohol	98.67
B	Imidazole	Heterocyclics	23.69
C	Pyridine	Heterocyclics	19.73
D	Cyclohexanol	Alcohol	8.03
E	Benzalkonium chloride (1%)	Cationic Surfactant	4.53
F	Sodium hydroxide (10%)	Alkalis	2.26

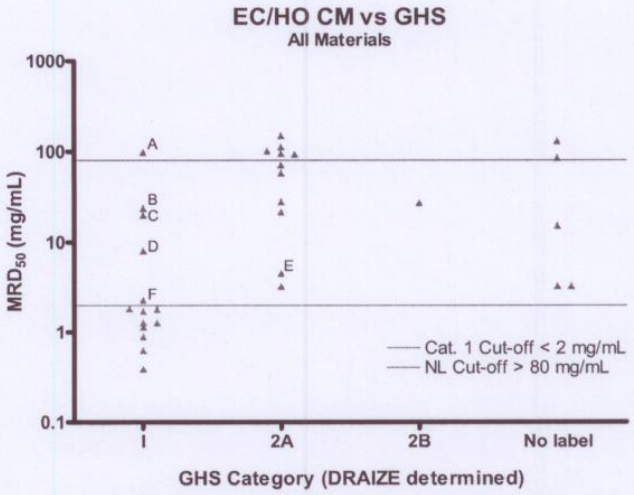


Figure 6.1.3.1.b Results of the EC/HO study related to GHS classification. Data points indicate the mean MRD_{50} for the laboratories (2, 3, or 4) that provided data for that chemical. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude. All materials including surfactants are included.

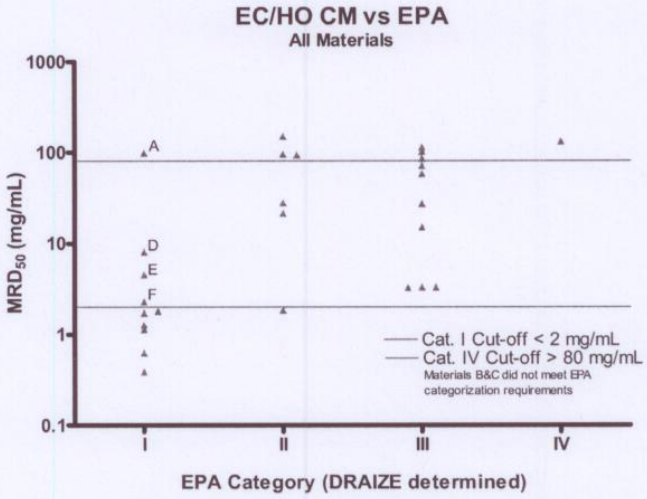


Figure 6.1.3.1.c Results of the EC/HO study related to EPA classification Data points indicate the mean MRD_{50} for the laboratories (2, 3, or 4) that provided data for that chemical. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude. All materials including surfactants are included.

Figures 6.1.3.1.a, 6.1.3.1.b, and 6.1.3.1c show cut-off values for MRD_{50} scores that have been empirically chosen to identify, where possible, the various hazard categories. In the case of the GHS system and the EPA system which have 4 categories, the overlap of MRD_{50} response was so large that it was deemed impossible to differentiate between the two middle categories. Hence only upper (to possible identify non-irritants) and lower (to possibly identify severe irritants) cut-off values are shown.

It appears from the graphs that the SM with the transwell protocol does not have the ability to clearly separate the wide range of test materials used in the EC/HO study into the Draize test defined EU, GHS or EPA categories. One exception is that severe irritants seem to be reasonably predicted when MRD_{50} scores of less than 2 are used. Using this lower cut-off value, there is a high positive predictive value for EU category R41 (100%; 9 of 9 chemicals), GHS category 1 (100%; 9 of 9) and EPA category I (86%; 6 of 7 chemicals).

Even though the positive predictive value was high using a lower cut-off of MRD_{50} <2 mg/ml, the sensitivity was lower, with several severe chemicals being under predicted in each hazard classification system. Each of these chemicals is identified on the Figures with a letter code defined in Table 6.1.3.1.b. Within these outliers are 2 of the 3 heterocyclics in the study, 2 of the 9 alcohols, 1 of 6 dilutions of cationic surfactants, and 1 strong alkali.

Contingency tables were created to determine the performance of the SM assay and the proposed cut-offs for each of the hazard classification systems. Results with the EU system are found in Table 6.1.3.1.c, results with the GHS system in Table 6.1.3.1.d and results with the EPA system in Table 6.1.3.e. In all of the cases the proposed cut-off for the most irritating categories resulted in a high positive predictive value (100% for the EU and GHS systems; 85.7% for the EPA system). Predictivity for the less irritating materials was considerably lower.

Table 6.1.3.1.c EC/HO - Contingency table depicting the concordance and predictivity of the CM assay for EU hazard classifications when the cut-off values shown in Figure 6.1.3.1.a are applied. N = 31 materials.

Draize Determined EU Category	EU Category Predicted by CM						
	R41	R36	Not Classified	Total	Concordance	Toxicity Overpredicted	Toxicity Underpredicted
R41	9	5	1	15	60.0%	NA	40.0%
R36	0	6	3	9	66.7%	0.0%	33.3%
Not Classified	0	3	4	7	57.1%	42.9%	NA
Total	9	14	8	31	61.3%		
Predictivity	100.0%	42.9%	50.0%				
Category Underpredicted	NA	35.7%	50.0%				
Category Overpredicted	0.0%	21.4%	NA				

Table 6.1.3.1.d EC/HO - Contingency table depicting the concordance and predictivity of the CM assay for GHS hazard classifications when the cut-off values shown in Figure 6.1.3.1.b are applied.

Draize Determined GHS Category	GHS Category Predicted By CM							
	1	2A	2B	No Label	Total	Concordance	Toxicity Overpredicted	Toxicity Underpredicted
1	9	4		1	14	64.3%	NA	35.7%
2A	0	6		5	11	54.5%	0.0%	45.5%
2B	0	1		0	1	100.0%	0.0%	0.0%
No Label	0	3		2	5	40.0%	60.0%	NA
Total	9	14		8	31	58.1%		
Predictivity	100.0%	50.0%		25.0%				
Category Underpredicted	NA	28.6%		75.0%				
Category Overpredicted	0.0%	21.4%		NA				

Table 6.1.3.1.e EC/HO - Contingency table depicting the concordance and predictivity of the CM assay for EPA hazard classifications when the cut-off values shown in Figure 6.1.3.1.c are applied. N = 27 materials.

Draize Determined EPA Category	EPA Category Predicted By CM							
	I	II	III	IV	Total	Concordance	Toxicity Overpredicted	Toxicity Underpredicted
I	6	3		1	10	60.0%	NA	40.0%
II	1	2		3	6	33.3%	16.7%	50.0%
III	0	7		3	10	70.0%	0.0%	30.0%
IV	0	0		1	1	100.0%	0.0%	NA
Total	7	12		8	27	59.3%		
Predictivity	85.7%	75.0%		12.5%				
Category Underpredicted	NA	25.0%		87.5%				
Category Overpredicted	14.3%	0.0%		NA				

An additional analysis was conducted to identify the ability of the *in vitro* assay to make two different binary classifications: severe irritants versus the rest, and non-irritating materials versus the rest. Severe irritants were defined to be those materials falling in the highest category (EU R41, GHS Category 1, or EPA category I) for each of the classification systems. Non-irritating materials were defined as those falling in the lowest category (EU Not Classified, GHS No Category, and EPA IV) for each of the classification systems. These results are given in Table 6.1.3.1.f.

Table 6.1.3.1.f EC/HO - Concordance table for severe irritants versus the rest and non-irritants versus the rest. Cut-off values from Figures 6.1.3.1.a, 6.1.3.1.b, and 6.1.3.1.c were applied. N = 31 materials (EU & GHS) and N = 27 materials (EPA).

	Vs Rest													
	Concordance		Sensitivity		Specificity		Positive Predictivity		Negative Predictivity		False Positive Rate		False Negative Rate	
	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.
EU Severe irritants	81%	25/31	60%	9/15	100%	16/16	100%	9/9	73%	16/22	0%	0/16	40%	6/15
EU non-irritants	77%	24/31	83%	20/24	57%	4/7	87%	20/23	50%	4/8	43%	3/7	17%	4/24
GHS Severe irritants	84%	26/31	64%	9/14	100%	17/17	100%	9/9	77%	17/22	0%	0/17	36%	5/14
GHS non-irritants	71%	22/31	77%	20/26	40%	2/5	87%	20/23	25%	2/8	60%	3/5	23%	6/26
EPA Severe irritants	81%	22/27	60%	6/10	94%	16/17	86%	6/7	80%	16/20	6%	1/17	40%	4/10
EPA non-irritants	74%	20/27	73%	19/26	100%	1/1	100%	19/19	13%	1/8	0%	0/1	27%	7/26

It can be seen from the above table that positive predictivity was relatively high for most of the situations, but only with the identification of severe irritants was the negative predictivity also relatively high (73%, 77%, and 80% for the EU, GHS and EPA systems, respectively).

Surfactant analysis

Since in recent years the applicability domain of the SM or CM assay has become more narrowly defined to be limited to test materials that are completely water soluble, and since much of the SM and CM testing over that same time period has been surfactants and surfactant-containing products, it was decided to investigate only the surfactants (no surfactant-based formulations were in the data set) from the EC/HO chemical set. There were 11 surfactants which had data from two or more labs. Figures 6.1.3.1.d, 6.1.3.1.e, and 6.1.3.1.f show the results of that analysis relative to EU, GHS, and EPA classifications, respectively.

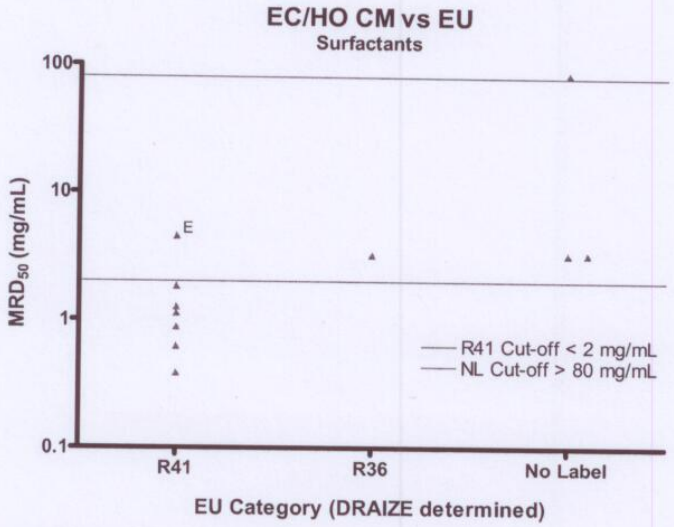


Figure 6.1.3.1.d Results of only the surfactants from the EC/HO study related to EU classification. Data points indicate the mean MRD_{50} for the laboratories (2, 3, or 4) that provided data for that chemical. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude.

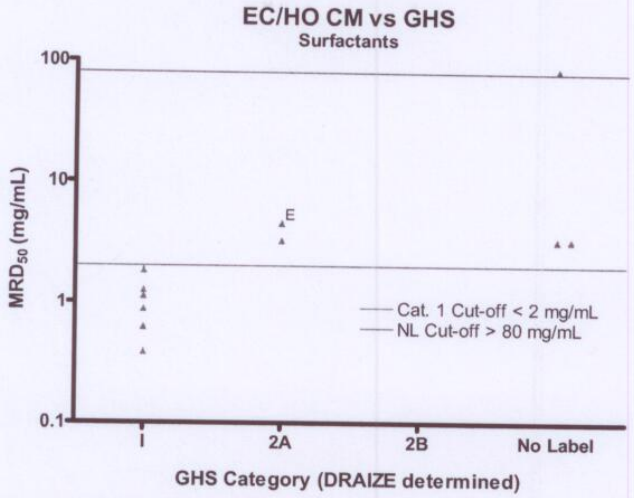


Figure 6.1.3.1.e Results of only the surfactants from the EC/HO study related to GHS classification. Data points indicate the mean MRD_{50} for the laboratories (2, 3, or 4) that provided data for that chemical. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude.

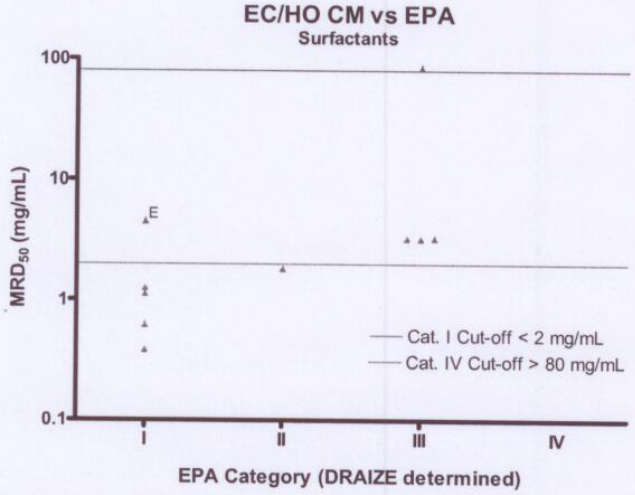


Figure 6.1.3.1.f Results of only the surfactants from the EC/HO study related to EPA classification. Data points indicate the mean MRD_{50} for the laboratories (2, 3, or 4) that provided data for that chemical. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude.

The smaller number of data points (11 for the EU and GHS analysis; 10 for the EPA analysis) make it difficult to set cut-off values based on these data sets alone. Therefore, we have kept the cut-offs used when analyzing the full range of chemicals, but it should be noted that these might not be optimal when a greater number of surfactants or surfactant-containing materials are assessed. Because of the limited amount of data, it is difficult to determine if the CM adequately separates the classifications for the EU, GHS, or EPA systems although (as with the complete set of chemicals) the lower cut-off of <2 mg/ml generally results in a high positive predictive value for R41 (6 of 6 materials; 100%), GHS 1's (6 of 6 materials; 100%), or EPA I's (4 of 5 materials; 80%). The one R41 and EPA I material which was under classified was 1% benzalkonium chloride.

Although a lower cut-off value may exist which separates EU Not Classified, GHS No Category or EPA Category IV from the other GHS, EU, or EPA Categories (we have hypothesized >80 mg/ml on Figures 6.1.3.1.d, 6.1.3.1.e, and 6.1.3.1.f), we believe there are insufficient data available from this study to make a definitive decision.

One significant difference between the surfactant analysis and the total chemical analysis is that the number of false negative materials is reduced significantly regardless of the hazard classification scheme. Comparing Figures 6.1.3.1.a, 6.1.3.1.b, and 6.1.3.1.c with Figures 6.1.3.1.d, 6.1.6.1.e, and 6.1.3.1.f shows that only material E (1% Benzalkonium Chloride) remains as an under predicted severe irritant in the EU or EPA classification systems.

Table 6.1.3.1.g EC/HO Surfactants - Contingency table depicting the concordance and predictivity of the CM assay for EU hazard classifications when the cut-off values shown in Figure 6.1.3.1.d are applied. N = 11 materials.

Draize Determined EU Category	EU Category Predicted by CM						
	R41	R36	Not Classified	Total	Concordance	Toxicity Overpredicted	Toxicity Underpredicted
R41	6	1	0	7	85.7%	NA	14.3%
R36	0	1	0	1	100.0%	0%	0%
Not Classified	0	2	1	3	33.3%	66.7%	NA
Total	6	4	1	11	72.7%		
Predictivity	100.0%	25.0%	100.0%				
Category Underpredicted	NA	25.0%	0%				
Category Overpredicted	0%	50.0%	NA				

Table 6.1.3.1.h EC/HO Surfactants - Contingency table depicting the concordance and predictivity of the CM assay for GHS hazard classifications when the cut-off values shown in Figure 6.1.3.1.e are applied. N = 11 materials.

Draize Determined GHS Category	GHS Category Predicted By CM							
	1	2A	2B	No Label	Total	Concordance	Toxicity Overpredicted	Toxicity Underpredicted
1	6	0		0	6	100.0%	NA	0%
2A	0	2		0	2	100.0%	0%	0%
2B	0	0		0	0	NA	NA	NA
No Label	0	2		1	3	33.3%	66.7%	NA
Total	6	4		1	11	81.8%		
Predictivity	100.0%	50.0%		100.0%				
Category Underpredicted	NA	0%		0%				
Category Overpredicted	0%	50.0%		NA				

Table 6.1.3.1.i EC/HO - SurfactantsContingency table depicting the concordance and predictivity of the CM assay for EPA hazard classifications when the cut-off values shown in Figure 6.1.3.1.f are applied. N = 10 materials.

Draize Determined EPA Category	EPA Category Predicted By CM							
	I	II	III	IV	Total	Concordance	Toxicity Overpredicted	Toxicity Underpredicted
I	4	1		0	5	80.0%	NA	20.0%
II	1	0		0	1	0.0%	100.0%	0.0%
III	0	3		1	4	75.0%	0%	25.0%
IV	0	0		0	0	NA	NA	NA
Total	5	4		1	10	70.0%		
Predictivity	80.0%	75.0%		0.0%				
Category Underpredicted	NA	25.0%		100.0%				
Category Overpredicted	20.0%	0%		NA				

Table 6.1.3.1.j EC/HO Surfactants - Concordance table for severe irritants versus the rest and non-irritants versus the rest. Cut-off values from Figures 6.1.3.1.d, 6.1.3.1.e, and 6.1.3.1.f were applied. N = 11 materials (EU & GHS) and N = 10 materials (EPA)

	Vs Rest													
	Concordance		Sensitivity		Specificity		Positive Predictivity		Negative Predictivity		False Positive Rate		False Negative Rate	
	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.
EU Severe irritants	91%	10/11	86%	6/7	100%	4/4	100%	6/6	80%	4/5	0%	0/4	14%	1/7
EU non-irritants	82%	9/11	100%	8/8	33%	1/3	80%	8/10	100%	1/1	67%	2/3	0%	0/8
GHS Severe irritants	100%	11/11	100%	6/6	100%	5/5	100%	6/6	100%	5/5	0%	0/5	0%	0/6
GHS non-irritants	82%	9/11	100%	8/8	33%	1/3	80%	8/10	100%	1/1	67%	2/3	0%	0/8
EPA Severe irritants	80%	8/10	80%	4/5	80%	4/5	80%	4/5	80%	4/5	20%	1/5	20%	1/5
EPA non-irritants	90%	9/10	90%	9/10	NA	0/0	100%	9/9	0%	0/1	NA	0/0	10%	1/10

Table 6.1.3.1.j presents the two binary classification systems of Severe Irritants versus the rest, and Non-Irritants versus the rest. The performance for all situations appears much better for the surfactants than for the analysis with all chemicals; however, there were only 11 data points in the surfactant analysis, while there were 28 data points in the total chemical analysis. In the case of the GHS severe irritants versus the rest there was both 100% positive predictive value and 100% negative predictive value. The analysis for EU non-irritants versus the rest, and GHS non-irritants versus the rest resulted in 100% negative predictive value. Using such a test to identify negative materials so they did not have to be animal tested would be helpful.

6.1.3.2 Analysis of the CTFA Phase III study (Gettings, Lordo et al. 1996)

The reference data for the CTFA Phase III study (Annex I) are arguably the most useful of the animal data used for any of the studies in this BRD. They were all obtained under GLP-compliant conditions and with a randomized block design utilizing three male and three female rabbits for each chemical. There are several advantages to the block design: 1) it simulates to some extent within lab day-to-day variability since for each chemical not all rabbits are dosed on the same day, and 2) it eliminates some of the scoring bias since the scorers read each animal independently and are unaware of which six rabbits were treated with the same test article. However, the main positive point about the study is that the *in vitro* and *in vivo* assays were run nearly currently (separated only by a few weeks) using samples from the same batch of chemical or formulation. The one negative point to this study is that ocular anesthesia was used during the rabbit test. Anesthesia is generally not used when conducting the Draize test, so this set of animal data is not completely compatible with the reference data for most of the other studies addressed in this BRD (see Section 6.1.3.4 for additional discussion).

A single laboratory (Company # 4) contributed SM data (Annex F3) for this study. All 25 chemicals in the study were deemed compatible for testing with the SM. An overall summary of the CTFA Phase III study including the chemical identities, animal scores, and *in vitro* scores are given in Table 6.1.3.2.a. Since these studies were conducted with the SM, for ease of comparison with the other studies in this section of the BRD, the *in vitro* MRD_{50} values have been converted to CM values using the relationship presented in section 2.2.1.

Table 6.1.3.2.a Summary of CTFA Phase III study (Gettings, Lordo et al. 1996). N = 25 materials.

***In Vivo* Eye Irritation Classifications - CTFA Phase III Study**

CTFA chemical number	Substance	Test Code	CASRN[1]	Concentration Tested	Purity (%)	n. of animals	*In Vivo* EU[2,3]	*In Vivo* GHS[4,5]	*In Vivo* EPA[6,7]	MMAS	CM converted value MRD_{50} (mg/mL)
1	Shampoo 7	HZA	NA	100%	NA	6	R41	Category 1	Category I	37.8	1.18
2	Liquid Soap 1	HZB	NA	25%	NA	6	Not classified	No category	Category III	20.7	2.80
3	Shampoo 1	HZC	NA	25%	NA	6	Not classified	No category	Category III	36.0	1.72
4	Shampoo 5	HZD	NA	25%	NA	6	Not classified	No category	Category III	19.5	2.78
5	Gel Cleanser	HZE	NA	100%	NA	6	R41	No category	Category I	22	3.19
6	Baby Shampoo 2	HZF	NA	100%	NA	6	R41	Category 1	Category I	37.5	1.50
7	Shampoo 8	HZG	NA	25%	NA	6	Not classified	No category	Category III	17.8	2.80
8	Eye Makeup re.	HZH	NA	100%	NA	6	Not classified	No category	Category IV	2.3	20.0
9	Skin Cleaner	HZI	NA	100%	NA	6	R41	Category 1	Category I	41.0	1.09
10	Mild Shampoo	HZJ	NA	100%	NA	6	Not classified	No category	Category IV	8.2	6.38
11	Bubble bath	HZK	NA	100%	NA	6	R41	Category 1	Category I	39.7	0.97
12	Foam Bath	HZL	NA	100%	NA	6	R41	Category 1	Category I	37.8	1.09
13	Shampoo 3	HZM	NA	25%	NA	6	Not classified	No category	Category III	12.7	3.11
14	Shampoo 6	HZN	NA	25%	NA	6	Not classified	No category	Category III	18.0	2.56
15	Baby Shampoo 1	HZP	NA	100%	NA	6	Not classified	No category	Category III	11.7	2.45
16	Cleansing Gel	HZQ	NA	100%	NA	6	Not classified	No category	Category III	17.2	5.85
17	Facial Cleansing Foam	HZR	NA	25%	NA	6	R41	No category	Category I	39.0	5.60
18	Shower Gel	HZS	NA	100%	NA	6	R41	Category 1	Category I	41.4	1.13
19	Polishing Scrub	HZT	NA	100%	NA	6	Not classified	No category	Category IV	7.0	30.9
20	Hand Soap	HZU	NA	25%	NA	6	Not classified	No category	Category III	33.7	4.85
21	Shampoo 4	HZV	NA	25%	NA	6	Not classified	No category	Category III	25.2	2.34
22	Liquid Soap 2	HZW	NA	25%	NA	6	Not classified	2B	Category III	31.0	2.64
23	Shampoo 2	HZX	NA	100%	NA	6	R41	Category 1	Category I	40.0	1.20
24	Shampoo AntiD	HZY	NA	100%	NA	6	R41	Category 1	Category I	43.0	1.14
25	Facial Cleanser	HZZ	NA	100%	NA	6	Not classified	No category	Category IV	3.7	>168.9

[1]CASRN=Chemical Abstract Services Registry Number

[2]EU=European Union (EU [2001]).

[3]Risk phrase R41 = risk of serious damage to the eyes; R36 = irritating to the eyes; not classified.

[4]GHS=Globally Harmonized System (UN [2003])

[5]Eye Irritant Category 1 = irreversible effects on the eye/serious damage to the eye; Category 2A = reversible effects on the eye/irritating to the eyes; Category 2B = reversible effects on the eye/mildly irritating to the eyes; No category

[6]EPA=U.S. Environmental Protection Agency (EPA [1996]).

[7]Toxicity Category I for the Primary Eye Irritation Study = Corrosive, or corneal involvement or irritation not reversible within 21 days; Category II = Corneal involvement or irritation clearing in 8-21 days; Category III = Corneal involvement or irritation clearing in 1-7 daysl; Category IV: minimal effects clearing in less than 24 hr

[9]MMAS scores reported in Gettings et al. (1996)

[9]SCNM=Study Crtieria Not Met

[10]n.p.=not provided

[11] NA = not applicable

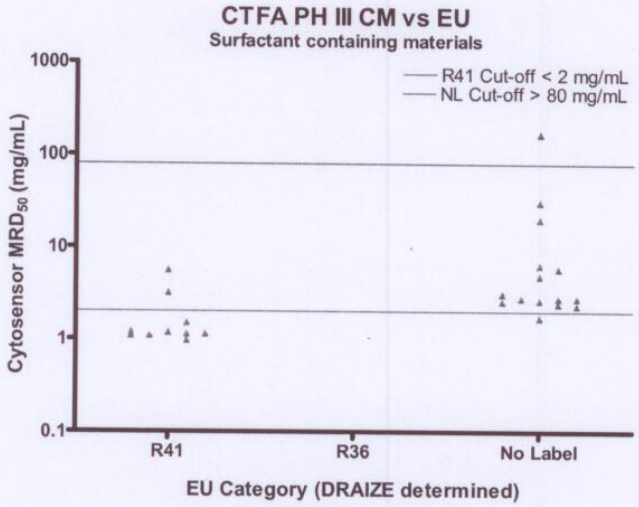


Figure 6.1.3.2.a Results of the CTFA Phase III study related to EU classification. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude. MRD_{50} is reported in converted CM values.

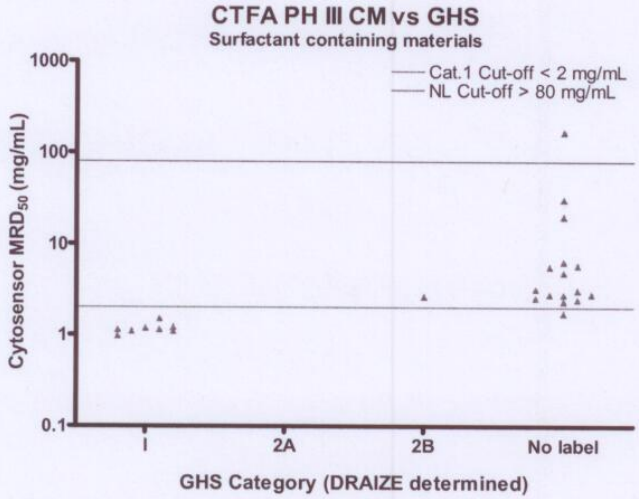


Figure 6.1.3.2.b Results of the CTFA Phase III study related to GHS classification. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude. MRD_{50} is reported in converted CM values.

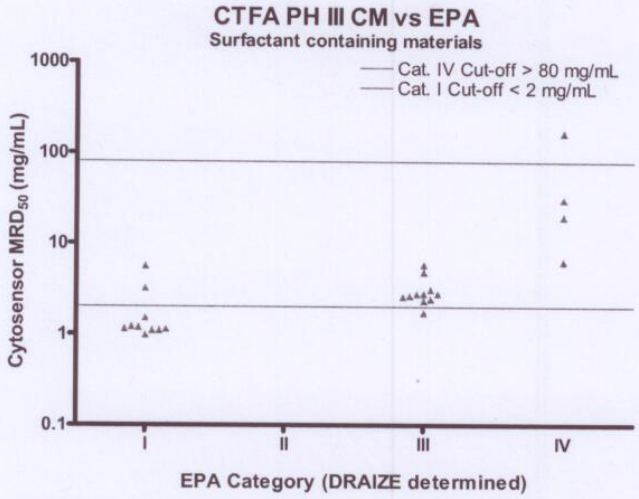


Figure 6.1.3.2.c Results of the CTFA Phase III study related to EPA classification. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude. MRD_{50} is reported in converted CM values.

Figures 6.1.3.2.a, 6.1.3.2.b, and 6.1.3.2.c show cut-off values for MRD_{50} scores that have been empirically chosen to identify, where possible, the various hazard categories. In attempting to select cut-off values we first tried those that were chosen from the EC/HO study (see preceding section). Since these appeared adequate, we continued the analysis with these values for the sake of consistency. As with the EC/HO study, in the case of the GHS system and the EPA system which have 4 categories, the overlap of MRD_{50} response was so large that it was deemed impossible to differentiate between the two middle categories. This analysis was made even more difficult because of the distribution of the hazard classifications. There were no R36 materials and only 1 GHS 2A or 2B material. Hence only upper (to possibly identify non-irritants) and lower (to possibly identify severe irritants) cut-off values are shown.

It appears from the graphs that the SM does not have the ability to clearly separate the surfactant-containing materials used in the CTFA Phase III study into the Draize test defined EU, GHS or EPA categories. One exception is that severe irritants seem to be reasonably predicted when MRD_{50} scores of less than 2 are used. Using this lower cut-off value, there is a high positive predictive value for EU category R41 (89%; 8 of 9 materials), GHS category 1 (89%; 8 of 9 materials) and EPA category I (89%; 8 of 9 materials).

Even though the positive predictive value was high using a lower cut-off of MRD_{50} <2 mg/ml, the sensitivity was lower, with several severe chemicals being under predicted by the EU and EPA classification system. Over predictions of mild materials (EU Not Classified, GHS No Label, and EPA IV) were very frequent in this study.

A more detailed analysis of the performance of the selected cut-off values is given in Tables 6.1.3.2.b, 6.1.3.2.c, and 6.1.3.2.d for the EU, GHS and EPA classification systems, respectively.

All materials in the CTFA Phase III study were surfactant-containing materials, so no additional analysis of subsets of the test materials was necessary.

Table 6.1.3.2.b CTFA - Contingency table depicting the concordance and predictivity of the CM assay for EU hazard classifications when the cut-off values shown in Figure 6.1.3.2.a are applied. N = 25 materials.

Draize Determined EU Category	EU Category Predicted by CM						
	R41	R36	Not Classified	Total	Concordance	Toxicity Overpredicted	Toxicity Underpredicted
R41	8	2	0	10	80.0%	NA	20.0%
R36	0	0	0	0	NA	NA	NA
Not Classified	1	13	1	15	6.7%	93.3%	NA
Total	9	15	1	25	36.0%		
Predictivity	88.9%	0.0%	100.0%				
Category Underpredicted	NA	13.3%	0%				
Category Overpredicted	11.1%	86.7%	NA				

Table 6.1.3.2.c CTFA - Contingency table depicting the concordance and predictivity of the CM assay for GHS hazard classifications when the cut-off values shown in Figure 6.1.3.2.b are applied. N = 25 materials.

Draize Determined GHS Category	GHS Category Predicted By CM							
	1	2A	2B	No Label	Total	Concordance	Toxicity Overpredicted	Toxicity Underpredicted
1	8	0		0	8	100.0%	NA	0%
2A	0	0		0	0	NA	NA	NA
2B	0	1		0	1	100.0%	0%	0%
No Label	1	14		1	16	6.3%	93.8%	NA
Total	9	15		1	25	44.0%		
Predictivity	88.9%	6.7%		100.0%				
Category Underpredicted	NA	0%		0%				
Category Overpredicted	11.1%	93.3%		NA				

Table 6.1.3.2.d CTFA - Contingency table depicting the concordance and predictivity of the CM assay for EPA hazard classifications when the cut-off values shown in Figure 6.1.3.2.c are applied. N = 25 materials.

Draize Determined EPA Category	EPA Category Predicted By CM							
	I	II	III	IV	Total	Concordance	Toxicity Overpredicted	Toxicity Underpredicted
I	8	2		0	10	80.0%	NA	20.0%
II	0	0		0	0	NA	NA	NA
III	1	10		0	11	90.9%	9.1%	0%
IV	0	3		1	4	25.0%	75.0%	NA
Total	9	15		1	25	76.0%		
Predictivity	88.9%	66.7%		100.0%				
Category Underpredicted	NA	13.3%		0%				
Category Overpredicted	11.1%	20.0%		NA				

An analysis of the ability of the SM assay to separate severe irritants from "the rest", and non-irritants from "the rest" is shown in Table 6.1.3.2.e. It can be seen that the SM assay performed quite well in some situations. For example when separating GHS severe irritants from "the rest", there was a 96% concordance, 89% positive predictivity and 100% negative predictivity. Also when separating EPA non-irritants from "the rest" there was an 88% concordance, an 88% positive predictivity and a 100% negative predictivity; however, there were only 4 non-irritants in the sample so the number of non-irritants would have to be increased before any significant conclusions could be drawn.

Table 6.1.3.2.e CTFA - Concordance table for severe irritants versus the rest and non-irritants versus the rest. Cut-off values from Figures 6.1.3.2.a, 6.1.3.2.b, and 6.1.3.2.c were applied. N = 25 materials.

	Vs Rest													
	Concordance		Sensitivity		Specificity		Positive Predictivity		Negative Predictivity		False Positive Rate		False Negative Rate	
	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.
EU Severe irritants	88%	22/25	80%	8/10	93%	14/15	89%	8/9	88%	14/16	7%	1/15	20%	2/10
EU non-irritants	44%	11/25	100%	10/10	7%	1/15	42%	10/24	100%	1/1	93%	14/15	0%	0/10
GHS Severe irritants	96%	24/25	100%	8/8	94%	16/17	89%	8/9	100%	16/16	6%	1/17	0%	0/8
GHS non-irritants	40%	10/25	100%	9/9	6%	1/16	38%	9/24	100%	1/1	94%	15/16	0%	0/9
EPA Severe irritants	88%	22/25	80%	8/10	93%	14/15	89%	8/9	88%	14/16	7%	1/15	20%	2/10
EPA non-irritants	88%	22/25	100%	21/21	25%	1/4	88%	21/24	100%	1/1	75%	3/4	0%	0/21

6.1.3.3 Analysis of the COLIPA study (Brantom, Bruner et al. 1997)

The reference data for the COLIPA study (Annex J) came from three main sources; two for the neat chemicals and one for the formulations. The data for the chemicals came from the ECETOC data bank (ECETOC 1992) and the EU isolated cornea study (Gautheron, Giroux et al. 1994). All of these data are now available in a new edition of the ECETOC data bank (ECETOC 1998). Twenty-three chemicals were used in this study, and 20 of the 23 are identical to – and use the same Draize values – as a portion of the chemicals used in the EC/HO study. All of these data were from historical studies.

Thirty-two formulations were used in the COLIPA study (Annex D11), and the Draize scores for these formulations come from Draize tests conducted contemporaneous with this study. The formulations were newly prepared for this study, but most were based on formulations that had been tested in Phases I, II, and III of the CTFA evaluation program (Feder, Lordo et al. 1991; Gettings, Dipasquale et al. 1994; Gettings, Lordo et al. 1996). Thus, it is likely that for the formulations, the *in vitro* tests were challenged with exactly the same material as the *in vivo* test. The same cannot be said for the chemicals since historical data were used for them.

Two laboratories (Company # 4 and Company # 5) contributed CM data for this study (Annexes F13 & H29). Company # 4 found that 29 of the 55 materials were compatible with the CM, while Company # 5 found that only 26 of 55 materials were compatible. Because of this, only the mean MRD_{50} values from the 26 materials where both laboratories provided data were used in this analysis. See section 5.2.3.2 for more details. An overall summary of the COLIPA study including the chemical identities, animal scores and *in vitro* scores (averages from Company # 4 and Company # 5) are given in Table 6.1.3.3.a.

Table 6.1.3.3.a Summary of the COLIPA study which includes average values from Company # 4 and Company # 5 laboratories (Brantom, Bruner et al. 1997). Surfactant materials are highlighted. N = 10 non-surfactant materials and N = 19 surfactant materials.

In Vivo Eye Irritation Classifications - COLIPA study

COLIPA chemical number	Substance	CASRN[1]	Concentration Tested	Purity (%)	n. of animals	*In Vivo* EU[2,3]	*In Vivo* GHS[4,5]	*In Vivo* EPA[6,7]	ECETOC MMAS Score[8]	Average MRD_{50} (mg/mL)
5	Shampoo no. 1 - normal		100%		3	R41	Category 1	Category 1	33.3	0.735
6	Eye make-up remover		100%		3	Not classified	No Category	Category IV	0.7	93.5
10	Imidazole		100%		3	R41	Category 1	SCNM	59.3	22.4
11	Polyethylene glycol 400		100%		6	Not classified	No Category	Category IV	0.0	306.4
12	Propylene glycol		100%		3	Not classified	No Category	Category IV	1.3	242.0
13	Triton X-100		1%		3	Not classified	No Category	Category III	1.7	19.0
14	Glycerol		100%		6	Not classified	No Category	Category IV	1.7	211.8
15	Tween 20		100%		4	Not classified	No Category	Category III	4.0	6.50
17	Sodium lauryl sulphate		3%		6	Not classified	No Category	Category III	16.0	3.00
18	Sodium hydroxide		1%		4	R36	Category 2B	Category III	25.8	11.34
19	Isopropanol		100%		4	Not classified	Category 2A	Category III	30.5	88.5
20	Triton X-100 [2]		5%		6	Not classified	Category 2A	Category III	32.3	3.54
21	Benzalkonium chloride [2]		1%		4	R41	Category 1	Category I	34.3	4.22
22	Methyl ethyl ketone		100%		6	R36	Category 2A	Category III	50.0	54.2*
23	Sodium lauryl sulphate		15%		6	R41	Category 1	Category I	59.2	0.513
24	Sodium lauryl sulphate		30%		6	R36	Category 2A	Category II	60.5	0.312*
25	Triton X-100		10%		6	R41	Category 1	Category II	59.0	1.85
26	Benzalkonium chloride		5%		4	R41	Category 1	Category I	83.8	1.095
27	Benzalkonium chloride		10%		3	R41	Category 1	Category I	108.0	0.314
28	Pump deodorant / antiperspirant		100%		3	Not classified	No Category	Category III	14.7	33.54
34	Gel cleanser		100%		3	Not classified	No Category	Category III	15.7	5.58
36	Shampoo - baby		100%		3	R41	Category 1	Category I	36.0	2.33
38	Hair styling lotion		100%		3	R36	Category 2A	Category III	19.3	228.4
39	Liquid soap no.1		100%		3	R41	Category 1	Category I	37.0	0.78
43	Mouthwash		100%		3	Not classified	No Category	Category IV	0.7	42.35
49	Skin cleanser		100%		3	R41	Category 1	Category I	34.3	0.70
52	Cetylpyridinium bromide		6%		4	R41	Category I	Category I	85.8	1.36*
54	Sodium hydroxide		10%		3	R41	Category 1	Category I	108.0	2.47
55	Trichloroacetic acid		30%		3	R41	Category 1	Category I	106.0	1.18

* - MA value only, CellTox AB designated unsuitable for testing, therefore, these values were not included in the analysis

[1]CASRN=Chemical Abstract Services Registry Number

[2]EU=European Union (EU [2001]).

[3]Risk phrase R41 = risk of serious damage to the eyes; R36 = irritating to the eyes; not classified

[4]GHS=Globally Harmonized System (UN [2003])

[5]Eye Irritant Category 1 = irreversible effects on the eye/serious damage to the eye; Category 2A = reversible effects on the eye/irritating to the eyes; Category 2B = reversible effects on the eye/mildly irritating to the eyes; No category

[6]EPA=U.S. Environmental Protection Agency (EPA [1996]).

[7]Toxicity Category I for the Primary Eye Irritation Study = Corrosive, or corneal involvement or irritation not reversible within 21 days; Category II = Corneal involvement or irritation clearing in 8-21 days; Category III = Corneal involvement or irritation clearing in 1-7 days; Category IV: minimal effects clearing in less than 24 hr

[8]MMAS scores reported in Brantom et al (1997)

[9]SCNM=Study Crtieria Not Met

[10]n.p =not provided

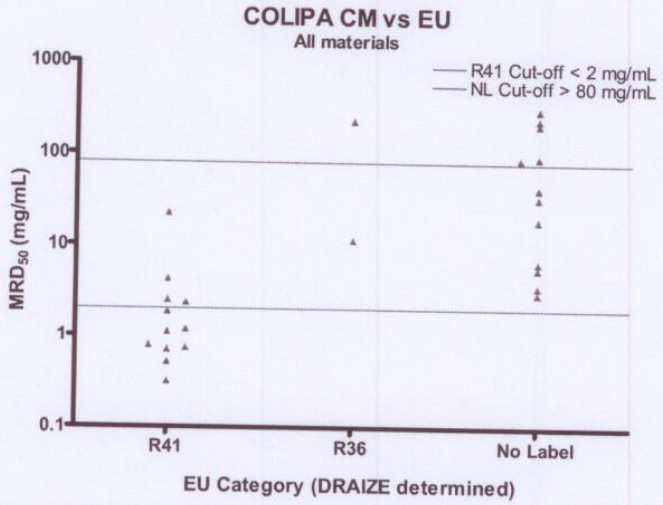


Figure 6.1.3.3.a Results of the COLIPA study related to EU classification. Data points indicate the mean MRD_{50} for both laboratories. Only data from test materials which were tested in both laboratories are shown here. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude.

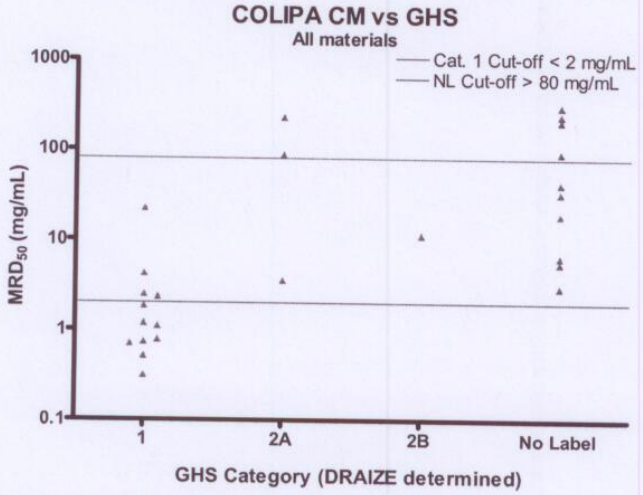


Figure 6.1.3.3.b Results of the COLIPA study related to GHS classification. Data points indicate the mean MRD_{50} for both laboratories. Only data from test materials which were tested in both laboratories are shown here. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude.

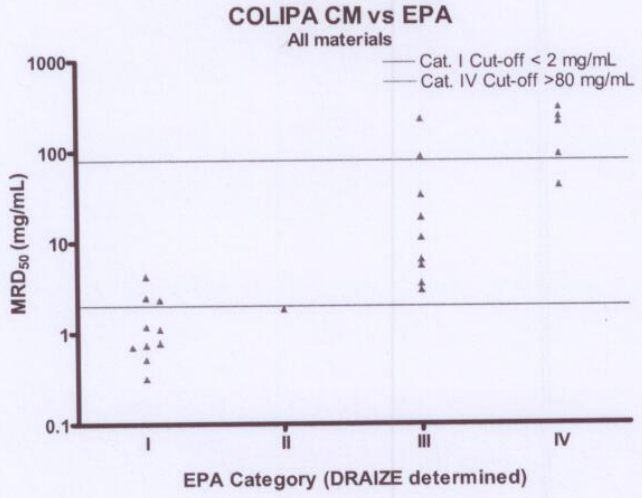


Figure 6.1.3.3.c Results of the COLIPA study related to EPA classification. Data points indicate the mean MRD_{50} for both laboratories. Only data from test materials which were tested in both laboratories are shown here. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude.

Figures 6.1.3.3.a, 6.1.3.3.b, and 6.1.3.3.c show cut-off values for MRD_{50} scores that have been empirically chosen to identify, where possible, the various hazard categories. In attempting to select cut-off values we first tried those that were chosen from the EC/HO study and CTFA Phase III studies (see preceding sections). Since these appeared adequate, we continued the analysis with these values for the sake of consistency. As with the EC/HO and CTFA Phase III studies, in the case of the GHS system and the EPA system which have 4 categories, the overlap of MRD_{50} response was so large that it was deemed impossible to differentiate between the two middle categories. This analysis was made even more difficult because of the distribution of the hazard classifications. There were only 2 R36 materials and only 4 GHS 2A or 2B materials. Hence only upper (to possibly identify non-irritants) and lower (to possibly identify severe irritants) cut-off values are shown.

It appears from the graphs that the CM does not have the ability to clearly separate the chemicals or surfactant-containing materials used in the COLIPA study into the Draize test defined EU, GHS or EPA categories. One exception is that severe irritants seem to be reasonably predicted when MRD_{50} scores of less than 2 are used. Using this lower cut-off value, there is a high positive predictive value for EU category R41 (100%; 8 of 8 materials), GHS category 1 (100%; 8 of 8 materials) and EPA category I (88%; 7 of 8 materials).

Even though the positive predictive value was high using a lower cut-off of MRD_{50} <2 mg/ml, the sensitivity was lower, with several severe chemicals being under predicted

by at least one hazard category by the EU, GHS, and EPA classification system. Over predictions of mild materials (EU Not Classified, GHS No Label, and EPA IV), especially by the EU and GHS system, occurred very frequent in this study.

Table 6.1.3.3.b COLIPA - Contingency table depicting the concordance and predictivity of the CM assay for EU hazard classifications when the cut-off values shown in Figure 6.1.3.3.a are applied. N = 26 materials.

Draize Determined EU Category	EU Category Predicted by CM						
	R41	R36	Not Classified	Total	Concordance	Toxicity over predicted	Toxicity under predicted
R41	8	4	0	12	66.7%	NA	33.3%
R36	0	1	1	2	50.0%	0%	50.0%
Not Classified	0	7	5	12	41.7%	58.3%	NA
Total	8	12	6	26	53.8%		
Predictivity	100.0%	8.3%	83.3%				
Category Underpredicted	NA	33.3%	16.7%				
Category Overpredicted	0.0%	58.3%	NA				

Table 6.1.3.3.c COLIPA - Contingency table depicting the concordance and predictivity of the CM assay for GHS hazard classifications when the cut-off values shown in Figure 6.1.3.3.b are applied. N = 26 materials.

Draize Determined GHS Category	GHS Category Predicted By CM							
	1	2A	2B	No Label	Total	Concordance	Toxicity over predicted	Toxicity under predicted
1	8	4		0	12	66.7%	NA	33.3%
2A	0	1		2	3	33.3%	0%	66.7%
2B	0	1		0	1	100.0%	0%	0%
No Label	0	6		4	10	40.0%	60.0%	NA
Total	8	12		6	26	53.8%		
Predictivity	100.0%	16.7%		66.7%				
Category Under predicted	NA	33.3%		33.3%				
Category Overpredicted	0%	50.0%		NA				

Table 6.1.3.3.d COLIPA - Contingency table depicting the concordance and predictivity of the CM assay for EPA hazard classifications when the cut-off values shown in Figure 6.1.3.3.c are applied. N = 25 materials.

Draize Determined EPA Category	EPA Category Predicted By CM							
	I	II	III	IV	Total	Concordance	Toxicity Overpredicted	Toxicity Underpredicted
I	7	3		0	10	70.0%	NA	30.0%
II	1	0		0	1	0.0%	100.0%	0%
III	0	7		2	9	77.8%	0.0%	22.2%
IV	0	1		4	5	80.0%	20.0%	NA
Total	8	11		6	25	72.0%		
Predictivity	87.5%	63.6%		66.7%				
Category Underpredicted	NA	27.3%		33.3%				
Category Overpredicted	12.5%	9.1%		NA				

An analysis of the ability of the CM assay to separate severe irritants from "the rest", and non-irritants from "the rest" is shown in Table 6.1.3.3.e. In general, the CM assay did not perform as well with this set of materials (a combination of surfactants and non-surfactant materials) as it did in the COLIPA study. In no case was there both a high positive predictivity and a high negative predictivity.

Table 6.1.3.3.e COLIPA - Concordance table for severe irritants versus the rest and non-irritants versus the rest. Cut-off values from Figures 6.1.3.3.a, 6.1.3.3.b, and 6.1.3.3.c were applied. N = 26 materials (EU & GHS) and N = 25 materials (EPA)

	Vs Rest													
	Concordance		Sensitivity		Specificity		Positive Predictivity		Negative Predictivity		False Positive Rate		False Negative Rate	
	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.
EU Severe irritants	85%	22/26	67%	8/12	100%	14/14	100%	8/8	78%	14/18	0%	0/14	33%	4/12
EU non-irritants	69%	18/26	93%	13/14	42%	5/12	65%	13/20	83%	5/6	58%	7/12	7%	1/14
GHS Severe irritants	85%	22/26	67%	8/12	100%	14/14	100%	8/8	78%	14/18	0%	0/14	33%	4/12
GHS non-irritants	69%	18/26	88%	14/16	40%	4/10	70%	14/20	67%	4/6	60%	6/10	13%	2/16
EPA Severe irritants	84%	21/25	70%	7/10	93%	14/15	88%	7/8	82%	14/17	7%	1/15	30%	3/10
EPA non-irritants	88%	22/25	90%	18/20	80%	4/5	95%	18/19	67%	4/6	20%	1/5	10%	2/20

Analysis of surfactants and surfactant-containing formulations

Since in recent years the applicability domain of the SM or CM assay has become more narrowly defined to be limited to test materials that are completely water soluble, and since much of the SM and CM testing over that same time period has been surfactants and surfactant-containing products, it was decided to investigate only the surfactants and surfactant-containing materials from the COLIPA test material set. There were 17 surfactants and surfactant-containing materials which had data from two labs. Figures 6.1.3.3.d, 6.1.3.3.e, and 6.1.3.3.f show the results of that analysis relative to EU, GHS, and EPA classifications, respectively.

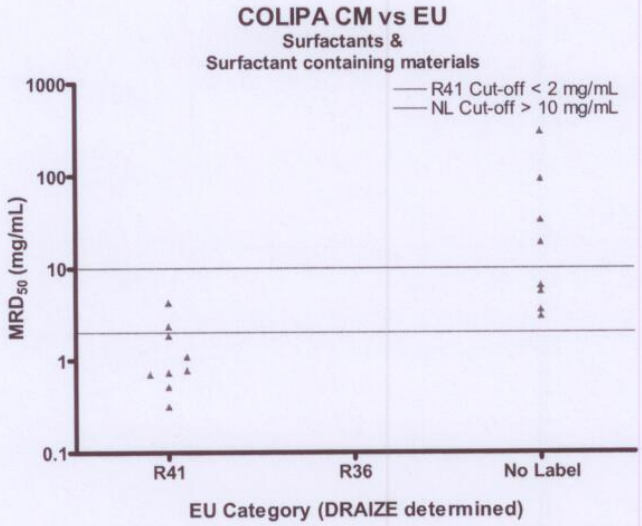


Figure 6.1.3.3.d Surfactant results of the COLIPA study related to EU classification. Data points indicate the mean MRD_{50} for both laboratories. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude.

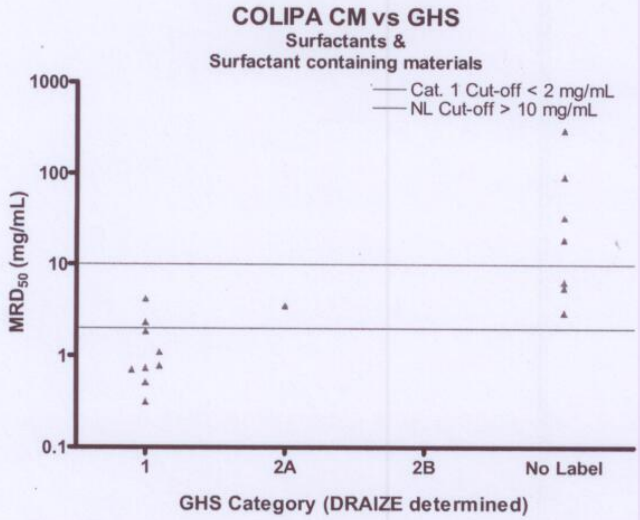


Figure 6.1.3.3.e Surfactant results of the COLIPA study related to GHS classification. Data points indicate the mean MRD_{50} for both laboratories. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude.

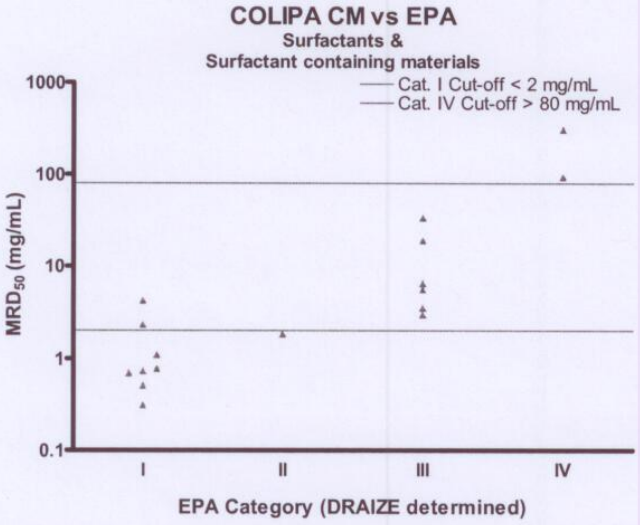


Figure 6.1.3.3.f Surfactant results of the COLIPA study related to EPA classification. Data points indicate the mean MRD_{50} for both laboratories. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude.

For these three data sets it appeared that a lower cut-off value (>10 mg/mL) than the previously used MRD_{50} > 80 mg/ml might be appropriate to identify the EU not classified and GHS no label from the more irritating. The cut-off of <2 mg/ml was retained for identifying R41, GHS 1 or EPA I materials. However, as seen in most of the previous analyses, there were very few materials in the EU R36, GHS 2A or 2B, or EPA II categories. This makes it difficult to determine exactly where the cut-off between these intermediate irritating categories and the mild categories lies. Additionally the EPA classification had only two Category IV materials, again making a decision for a cut-off almost impossible. To make the analysis even more difficult there were no R36 materials and only 1 GHS 2A or 2B materials. Hence only upper (to possibly identify non-irritants) and lower (to possibly identify severe irritants) cut-off values are shown on the three scatter plots.

It appears from the graphs that the CM does not have the ability to clearly separate the surfactants or surfactant-containing materials used in the COLIPA study into the Draize test defined EU, GHS or EPA categories. One exception is that severe irritants seem to be reasonably predicted when MRD_{50} scores of less than 2 are used. Using this lower cut-off value, there is a high positive predictive value for EU category R41 (100%; 7 of 7 materials), GHS category 1 (100%; 7 of 7 materials) and EPA category I (86%; 6 of 7 materials).

Even though the positive predictive value was high using a lower cut-off of MRD_{50} <2 mg/ml, the sensitivity was lower, with several severe chemicals being under predicted by at least one hazard category by the EU, GHS, and EPA classification system. Over predictions of mild materials (EU Not Classified, GHS No Label, and EPA IV), were not as great as found in the previous studies.

Table 6.1.3.3.f COLIPA Surfactant and surfactant containing materials - Contingency table for depicting the concordance and predictivity of the CM assay for EU hazard classifications when the cut-off values shown in Figure 6.1.3.3.d are applied. N = 17 surfactant materials.

Draize Determined EU Category	EU Category Predicted by CM						
	R41	R36	Not Classified	Total	Concordance	Toxicity Overpredicted	Toxicity Underpredicted
R41	7	2	0	9	77.8%	NA	22.2%
R36	0	0	0	0	NA	NA	NA
Not Classified	0	4	4	8	50.0%	50.0%	NA
Total	7	6	4	17	64.7%		
Predictivity	100.0%	0.0%	100.0%				
Category Underpredicted	NA	33.3%	0%				
Category Overpredicted	0%	66.7%	NA				

Table 6.1.3.3.g COLIPA Surfactant and surfactant containing materials - Contingency table depicting the concordance and predictivity of the CM assay for GHS hazard classifications when the cut-off values shown in Figure 6.1.3.3.e are applied. N = 17 surfactant materials.

Draize Determined GHS Category	GHS Category Predicted By CM							
	1	2A	2B	No Label	Total	Concordance	Toxicity Overpredicted	Toxicity Underpredicted
1	7	2		0	9	77.8%	NA	22.2%
2A	0	1		0	1	100.0%	0%	0%
2B	0	0		0	0	NA	NA	NA
No Label	0	3		4	7	57.1%	42.9%	NA
Total	7	6		4	17	70.6%		
Predictivity	100.0%	16.7%		100.0%				
Category Underpredicted	NA	33.3%		0%				
Category Overpredicted	0%	50.0%		NA				

Table 6.1.3.3.h COLIPA Surfactant and surfactant containing materials - Contingency table depicting the concordance and predictivity of the CM assay for EPA hazard classifications when the cut-off values shown in Figure 6.1.3.3.f are applied. N = 17 surfactant materials.

Draize Determined EPA Category	EPA Category Predicted By CM							
	I	II	III	IV	Total	Concordance	Toxicity Overpredicted	Toxicity Underpredicted
I	6	2		0	8	75.0%	NA	25.0%
II	1	0		0	1	0.0%	100.0%	0%
III	0	6		0	6	100.0%	0%	0%
IV	0	0		2	2	100.0%	0%	NA
Total	7	8		2	17	82.4%		
Predictivity	85.7%	75.0%		100.0%				
Category Underpredicted	NA	25.0%		0%				
Category Overpredicted	14.3%	NA		NA				

An analysis of the ability of the CM assay to test surfactants and surfactant-based materials and separate severe irritants from "the rest", and non-irritants from "the rest" is shown in Table 6.1.3.3.h. Although the assay seemed to perform well in some situations, e.g. when separating EPA non-irritants from "the rest" both the positive and negative predictivities were 100%, the fact that only 17 materials were included in the analysis prevents any strong conclusions from being drawn.

Table 6.1.3.3.i COLIPA Surfactant and surfactant containing materials - Concordance table for surfactant and surfactant containing matierlas for severe irritants versus the rest and non-irritants versus the rest. Cut-off values from Figures 6.1.3.3.d, 6.1.3.3.e, and 6.1.3.3.f were applied. N = 17 surfactant materials.

	Vs Rest													
	Concordance		Sensitivity		Specificity		Positive Predictivity		Negative Predictivity		False Posivite Rate		False Negative Rate	
	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.
EU Severe irritants	88%	15/17	78%	7/9	100%	8/8	100%	7/7	80%	8/10	0%	0/8	22%	2/9
EU non-irritants	76%	13/17	100%	9/9	50%	4/8	69%	9/13	100%	4/4	50%	4/8	0%	0/9
GHS Severe irritants	88%	15/17	78%	7/9	100%	8/8	100%	7/7	80%	8/10	0%	0/8	22%	2/9
GHS non-irritants	82%	14/17	100%	10/10	57%	4/7	77%	10/13	100%	4/4	43%	3/7	0%	0/10
EPA Severe irritants	82%	14/17	75%	6/8	89%	8/9	86%	6/7	80%	8/10	11%	1/9	25%	2/8
EPA non-irritants	100%	17/17	100%	15/15	100%	2/2	100%	15/15	100%	2/2	0%	0/2	0%	0/15

6.1.3.4 Analysis of the combined CTFA Phase III, EC/HO, and COLIPA data

Since each of the previously described studies had a relatively small number of data points – and the prediction models were being developed *post hoc* - we felt it would be more accurate to combine information from all three studies so that a more comprehensive prediction model(s) could be developed. At the same time we analyzed the data according to their distribution into the more defined applicability domains of surfactant materials, non-surfactant materials, surfactant-containing products (or mixtures) and non surfactant-containing products. Even so, the number of data points was still rather low, e.g. only 53 materials (not necessarily unique) were available when just the surfactants and surfactant-containing materials from the CTFA, EC/HO and COLIPA studies were considered. We justified combining data from these three studies with the knowledge that identical CM protocols were used for the EC/HO and COLIPA studies, and that the CTFA data could be converted from SM data to CM data.

The positive justifications for combining data notwithstanding, there are at least two caveats that should be considered in reviewing the analysis. First, the animal tests used to categorize the test materials differed slightly among the studies; topical ocular anesthesia was used for the CTFA studies but was not used for the EC/HO study or the COLIPA study. Secondly – and perhaps more importantly – the materials used in the three studies have some amount of overlap. However, the degree of overlap involved is not clear. Specifically, the COLIPA study attempted to use six of the surfactant formulations that were used in the CTFA study, but the test materials had to be reformulated for the COLIPA study which took place several years later. Thus it is likely that the formulations were very similar, but probably slightly different. Also seven surfactant/concentration combinations were duplicated between the EC/HO study and the COLIPA study, but there is no evidence that the materials were truly identical since they may have been procured from different sources and at different purities.

Therefore, we have chosen to graph all of the data points from the combined studies and use them to determine the prediction models and the performance statistics; but in each of these cases the number of truly unique materials is listed. In addition we also list the number of unique chemicals since some of the chemicals were tested at several different concentrations.

Table 6.1.3.4.a Surfactant Chemicals from the EC/HO, COLIPA, and CTFA studies. There are 21 total materials; 13 unique chemical:concentration combinations, and 6 totally unique surfactants. N = 21 surfactant chemicals.

Substance	Concentration Tested	Purity (%)	no. of animals	In Vivo EU[2,3]	In Vivo GHS[4,5]	In Vivo EPA[6,7]	ECETOC MMAS Score[8]	MRD_{50} (mg/mL) [11]	Protocol
Benzalkonium chloride	5		4	R41	Category 1	Category I	83.75	1.1	COLIPA
Benzalkonium chloride	10		3	R41	Category 1	Category I	108	0.31	COLIPA
Benzalkonium chloride (1%) (1x)	1	98	4	R41	Category 2A	Category I	34.4	4.53	EC/HO
Benzalkonium chloride (10%)	10	98	3	R41	Category 1	Category I	108	0.39	EC/HO
Benzalkonium chloride (5%)	5	98	4	R41	Category 1	Category I	83.8	1.13	EC/HO
Benzalkonium chloride [2]	1		4	R41	Category 1	Category I	34.25	4.22	COLIPA
Cetylpyridinium bromide (0.1%)	0.1	99	6	Not classified	No Category	Category III	2.7	84.65	EC/HO
Cetylpyridinium bromide (10%)	10	99	6	R41	Category 1	Category I	89.7	1.26	EC/HO
Cetylpyridinium bromide (6%)	6	99	4	R41	Category 1	SCNM[9]	85.8	0.87	EC/HO
Polyethylene glycol 400	100		6	Not classified	No Category	Category IV	0	306.36	COLIPA
Sodium lauryl sulfate (15%)	15	98	6	R41	Category 1	Category I	59.2	0.62	EC/HO
Sodium lauryl sulfate (3%)	3	98	6	Not classified	No Category	Category III	16	3.28	EC/HO
Sodium lauryl sulphate	3		6	Not classified	No Category	Category III	16	3	COLIPA
Sodium lauryl sulphate	15		6	R41	Category 1	Category I	59.17	0.51	COLIPA
Triton X-100	1		3	Not classified	No Category	Category III	1.67	18.98	COLIPA
Triton X-100	10		6	R41	Category 1	Category II	59	1.85	COLIPA
Triton X-100 (10%)	10	98	6	R41	Category 1	Category II	68.7	1.82	EC/HO
Triton X-100 (5%) (1x)	5	98	6	R36	Category 2A	Category III	32.3	3.23	EC/HO
Triton X-100 [2]	5		6	Not classified	Category 2A	Category III	32.33	3.54	COLIPA
Tween 20	100	98	4	Not classified	No Category	Category III	4	3.27	EC/HO
Tween 20	100		4	Not classified	No Category	Category III	4	6.5	COLIPA

Table 6.1.3.4.b Surfactant Formulations from the EC/HO, COLIPA, and CTFA studies. There are 32 test materials; most likely all are unique. N = 32 surfactant formulations.

Substance	Concentration Tested	Purity (%)	no. of animals	In Vivo EU[2,3]	In Vivo GHS[4,5]	In Vivo EPA[6,7]	ECETOC MMAS Score[8]	MRD_{50} (mg/mL) [11]	Protocol
Baby Shampoo 1	100	NA	6	Not classified	No category	Category III	11.7	2.45	CTFA
Baby Shampoo 2	100	NA	6	R41	Category 1	Category I	37.5	1.5	CTFA
Bubble bath	100	NA	6	R41	Category 1	Category I	39.7	0.97	CTFA
Cleansing Gel	100	NA	6	Not classified	No category	Category III	17.2	5.85	CTFA
Eye Makeup re.	100	NA	6	Not classified	No category	Category IV	2.3	20.03	CTFA
Eye make-up remover	100		3	Not classified	No Category	Category IV	0.67	93.54	COLIPA
Facial Cleanser	100	NA	6	Not classified	No category	Category IV	3.7	>168.9	CTFA
Facial Cleansing Foam	25	NA	6	R41	Category 2A	Category I	39	5.6	CTFA
Foam Bath	100	NA	6	R41	Category 1	Category I	37.8	1.09	CTFA
Gel cleanser	100		3	Not classified	No Category	Category III	15.67	5.58	COLIPA
Gel Cleanser	100	NA	6	R41	No category	Category I	22	3.19	CTFA
Hand Soap	25	NA	6	Not classified	No category	Category III	33.7	4.85	CTFA
Liquid Soap 1	25	NA	6	Not classified	No category	Category III	20.7	2.8	CTFA
Liquid Soap 2	25	NA	6	Not classified	Category 2B	Category III	31	2.64	CTFA
Liquid soap no.1	100		3	R41	Category 1	Category I	37	0.78	COLIPA
Mild Shampoo	100	NA	6	Not classified	No category	Category IV	8.2	6.38	CTFA
Polishing Scrub	100	NA	6	Not classified	No category	Category IV	7	30.85	CTFA
Pump deodorant / antiperspirant	100		3	Not classified	No Category	Category III	14.67	33.54	COLIPA
Shampoo – baby	100		3	R41	Category 1	Category I	36	2.33	COLIPA
Shampoo 1	25	NA	6	Not classified	No category	Category III	36	1.72	CTFA
Shampoo 2	100	NA	6	R41	Category 1	Category I	40	1.2	CTFA
Shampoo 3	25	NA	6	Not classified	No category	Category III	12.7	3.11	CTFA
Shampoo 4	25	NA	6	Not classified	No category	Category III	25.2	2.34	CTFA
Shampoo 5	25	NA	6	Not classified	No category	Category III	19.5	2.78	CTFA
Shampoo 6	25	NA	6	Not classified	No category	Category III	18	2.56	CTFA
Shampoo 7	100	NA	6	R41	Category 1	Category I	37.8	1.18	CTFA
Shampoo 8	25	NA	6	Not classified	No category	Category III	17.8	2.8	CTFA
Shampoo AntiD	100	NA	6	R41	Category 1	Category I	43	1.14	CTFA
Shampoo no. 1 – normal	100		3	R41	Category 1	Category I	33.33	0.74	COLIPA
Shower Gel	100	NA	6	R41	Category 1	Category I	41.4	1.13	CTFA
Skin Cleaner	100	NA	6	R41	Category 1	Category I	41	1.09	CTFA
Skin cleanser	100		3	R41	Category 1	Category I	34.33	0.7	COLIPA

Table 6.1.3.4.c Non-surfactant Chemicals from the EC/HO, COLIPA, and CTFA studies. There are 27 total materials; 21 unique chemical:concentration combinations, and 19 totally unique chemicals. N = 27 non-surfactant chemicals.

Substance	Concentration Tested (%)	Purity (%)	no. of animals	In Vivo EU[2,3]	In Vivo GHS[4,5]	In Vivo EPA[6,7]	ECETOC MMAS Score[8]	MRD_{50} (mg/mL) [11]	Protocol
2,5-Dimethylhexanediol	100	99.5	3	R41	Category 1	Category I	28.3	98.67	EC/HO
Acetone	100	99	4	R36	Category 2A	Category II	65.8	148.82	EC/HO
Ammonium nitrate	100	99.999	3	R36	Category 2A	Category III	18.3	71.27	EC/HO
Cyclohexanol	100	97	4	R41	Category 1	Category I	79.8	8.03	EC/HO
Ethanol	100	n.p.	3	Not classified	Category 2A	Category III	24	111.99	EC/HO
Gammabutyrolactone	100	>99	3	R36	Category 2A	Category II	43	93.79	EC/HO
Glycerol	100	>99.5	6	Not classified	No category	Category IV	1.7	129.88	EC/HO
Glycerol	100		6	Not classified	No Category	Category IV	1.7	211.77	COLIPA
Imidazole	100	99	3	R41	Category 1	SCNM	59.3	23.69	EC/HO
Imidazole	100		3	R41	Category 1	SCNM	59.3	22.43	COLIPA
Isobutanol	100	99.9	4	R36	Category 2A	Category II	60.3	27.91	EC/HO
Isopropanol	100	99.9	4	Not classified	Category 2A	Category III	30.5	101.26	EC/HO
Isopropanol	100		4	Not classified	Category 2A	Category III	30.5	88.55	COLIPA
Methyl acetate	100	98	4	R36	Category 2A	Category II	39.5	94.68	EC/HO
Methyl cyanoacetate	100	99	3	R36	Category 2A	Category II	27.7	21.54	EC/HO
Methyl ethyl ketone	100	99	4	R36	Category 2A	Category III	50	58.08	EC/HO
Promethazine HCl	100	98	3	R41	Category 1	SCNM	71.7	1.27	EC/HO
Propylene glycol	100		3	Not classified	No Category	Category IV	1.3	241.97	COLIPA
Pyridine	100	>99.9	3	R41	Category 1	SCNM	48	19.73	EC/HO
Sodium hydroxide	1		4	R36	Category 2B	Category III	25.8	11.34	COLIPA
Sodium hydroxide (1%)	1	reagent grade	4	R36	Category 2B	Category III	25.8	27.04	EC/HO
Sodium hydroxide	10		3	R41	Category 1	Category I	108	2.47	COLIPA
Sodium hydroxide (10%)	10	reagent grade	1	R41	Category 1	Category I	108	2.26	EC/HO
Sodium perborate, 4H2O	100	98.6	6	R41	Category 1	Category I	30.5	1.69	EC/HO
Trichloroacetic acid	30		3	R41	Category 1	Category I	106	1.18	COLIPA
Trichloroacetic acid (30%)	30	reagent grade	1	R41	Category 1	Category I	106	1.79	EC/HO
Trichloroacetic acid (3%)	3	reagent grade	6	Not classified	No category	Category III	6.7	15.03	EC/HO

Table 6.1.3.4.d Non-surfactant Formulations from the EC/HO, COLIPA, and CTFA studies. N = 2 non-surfactant formulations.

Substance	Concentration Tested	Purity (%)	no. of animals	In Vivo EU[2,3]	In Vivo GHS[4,5]	In Vivo EPA[6,7]	ECETOC MMAS Score[8]	MRD_{50} (mg/mL) [11]	Protocol
Hair styling lotion	100		3	R36	Category 2A	Category III	19.33	228.41	COLIPA
Mouthwash	100		3	Not classified	No Category	Category IV	0.67	42.35	COLIPA

We attempted to investigate the effect of topical ocular anesthesia on the Draize test results by comparing the 6 materials that were formulated to be identical between the CTFA and the COLIPA studies. This comparison is shown in Table 6.1.3.4.e. No clear cut pattern could be seen; for two materials the hazard categories were lower in the CTFA study, for one material the categories were higher, and for three materials the categories were the same. This discrepancy, combined with the fact that it was impossible to determine whether or not the formulations were identical since they were formulated several years apart, made it clear that it would be very difficult to determine the effect of topical anesthesia on the Draize score. This made it even more difficult to determine whether the six materials should be treated as identical; however we felt that it was not unreasonable to continue the evaluation of predictive capacity by treating the twelve data sets (six from the CTFA study, six from the COLIPA study) as if they had come from unique formulations.

Table 6.1.3.4.e Comparison of animal data for the CTFA and COLIPA studies. Liquid Soap 1 and Shampoo #1 used in the COLIPA study were formulated at 25% the strength used in the CTFA study, but were tested without dilution as was done in the CTFA study. Thus the actual tested concentrations of the formulations were the same.

CTFA								COLIPA							
Substance Designation	Conc. Tested	n. of animals	EU	GHS	EPA	MMAS	CM converted value MRD_{50} (mg/mL)	Substance Designation	Conc. Tested	n. of animals	EU	GHS	EPA	MMAS	Average MRD_{50} (mg/mL) (avg MA & CT)
Liquid Soap 1	25%	6	Not classified	No category	Category III	20.7	2.80	Liquid Soap 1	100%	3	R41	Category 1	Category I	37.0	0.78
Gel Cleanser	100%	6	R41	No category	Category I	22	3.19	Gel Cleanser	100%	3	Not classified	No Category	Category III	15.7	5.58
Baby Shampoo 2	100%	6	R41	Category 1	Category I	37.5	1.50	Shampoo - Baby	100%**	3	R41	Category 1	Category I	36.0	2.33
Shampoo 1	25%	6	Not classified	No category	Category III	36	1.72	Shampoo #1	100%**	3	R41	Category 1	Category I	33.3	0.735
Eye Makeup Remover	100%	6	Not classified	No category	Category IV	2.3	20.0	Eye Makeup Remover	100%	3	Not classified	No Category	Category IV	0.67	93.5
Skin Cleaner	100%	6	R41	Category 1	Category I	41	1.09	Skin Cleaner	100%	3	R41	Category 1	Category I	34.3	0.696

** - COLIPA formulation slightly different than the CTFA formulation

We next constructed scatter plots of the combined data for surfactants, surfactant containing formulations and non-surfactant materials plotted against the EU, GHS and EPA hazard categories in order to determine the appropriate cut-offs for the prediction model(s). Because only two materials were available in the non-surfactant formulations category, data for these materials were not plotted as a separate group, but were combined with the non-surfactant materials. Non-surfactant materials were also plotted by themselves. Finally the surfactant chemicals and the surfactant formulations were plotted together to determine if a single set of cut-off MRD_{50} values could be used for both.

6.1.3.4.1 Analysis of the combined CTFA, EC/HO, and COLIPA data with EU category

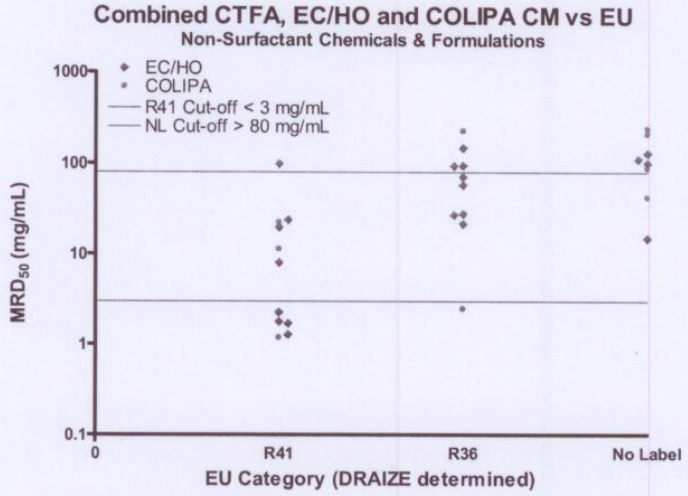


Figure 6.1.3.4.1.a Combined data for all non-surfactant containing chemicals and formulations from the CTFA, EC/HO and COLIPA studies graphed against EU hazard classifications. Proposed MRD_{50} cut-off values of >80 mg/ml to define No Label materials and <3 mg/ml to define Category R41 materials are shown. There are 29 total materials; 23 unique chemical:concentration combinations, and 21 totally unique chemicals.

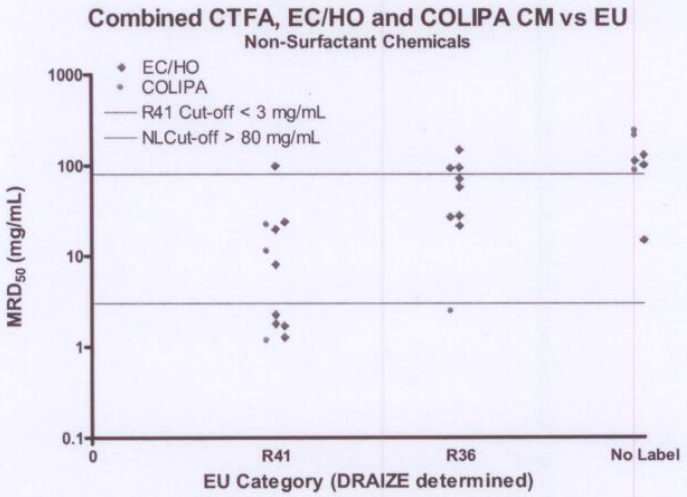


Figure 6.1.3.4.1.b Combined data for non-surfactant chemicals from the CTFA, EC/HO and COLIPA studies graphed against EU hazard classifications. Proposed MRD_{50} cut-off values of >80 mg/ml to define No Label materials and <3 mg/ml to define Category R41 materials are shown. There are 27 total materials; 21 unique chemical:concentration combinations, and 19 totally unique chemicals.

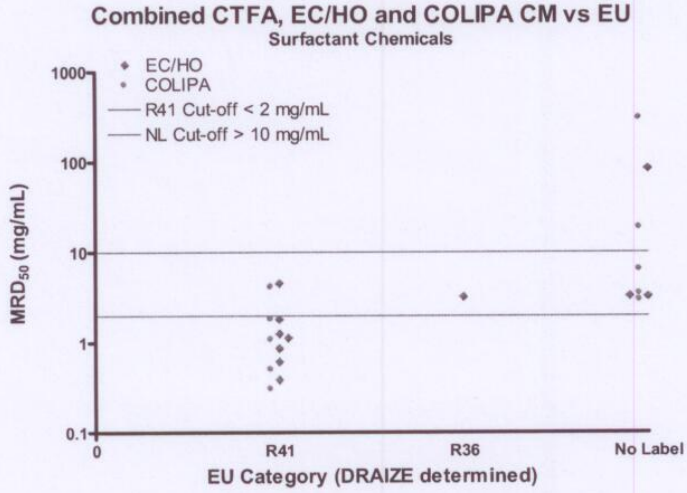


Figure 6.1.3.4.1.c Combined data for surfactant chemicals from the CTFA, EC/HO and COLIPA studies graphed against EU hazard classifications. Proposed MRD_{50} cut-off values of >10 mg/ml to define No Label materials and <2 mg/ml to define Category R41 materials are shown. There are 21 total materials; 13 unique chemical:concentration combinations, and 6 totally unique chemicals.

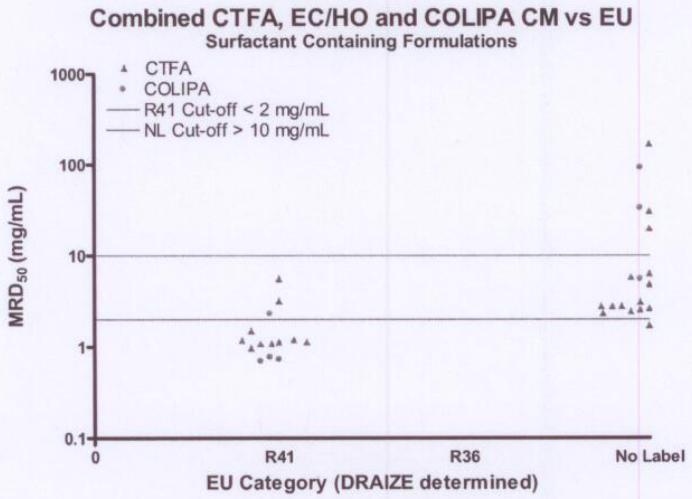


Figure 6.1.3.4.1.d Combined data for surfactant formulations from the CTFA, EC/HO and COLIPA studies graphed against EU hazard classifications. Proposed MRD_{50} cut-off values of >10 mg/ml to define No Label materials and <2 mg/ml to define Category R41 materials are shown. There are 32 totally unique chemicals. The assumption is that the six reformulated test materials for the COLIPA study are different from similar test materials used in the CTFA study.

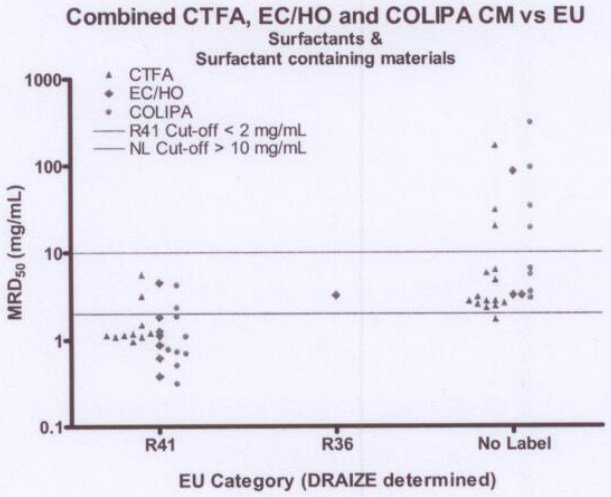


Figure 6.1.3.4.1.e Combined data for surfactants and surfactant containing materials from the CTFA, EC/HO and COLIPA studies graphed against EU hazard classifications. Proposed MRD_{50} cut-off values of >10 mg/ml to define No Label materials and <2 mg/ml to define R41 materials are shown. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude. There are 53 total materials; 45 unique chemical:concentration combinations, and 38 totally unique chemicals/formulations.

Starting with the EU hazard classification, a possible prediction model was determined empirically by trying to balance over predictions and under predictions in a conservative manner that kept under predictions to a minimum. For the combined non-surfactant chemicals and formulations, and non-surfactant chemicals alone (Figures 6.1.3.4.1.a and 6.1.3.4.1.b, this seemed to be done by cut-off values at 10 mg/ml and 3 mg/ml such that EU R41 predictions would be made for materials with MRD_{50} values ≤ 3.0 mg/ml, EU R36 predictions would be made for materials where their MRD_{50} was between 3 and 80 mg/ml and Not Classified for materials with MRD_{50} ≥ 80 mg/ml. Cut-off values are represented in the figures by horizontal lines. Some data points which overlapped have been displaced horizontally along the X-axis so that they are more easily visible. The predictive capacity for this prediction model for non-surfactant chemicals (there were only two non-surfactant formulations) is shown in Table 6.1.3.4.1.b. It can be seen from both the Table and the Figure 6.1.3.4.1.b that there is a high level of under prediction of both R41 (55%) and R36 (33%) categories.

A similar approach was taken for the surfactants and surfactant containing formulations. Figures 6.1.3.4.1.c and 6.1.2.4.1.d show that for these materials a slightly lower cut-off (2 mg/ml) was selected to identify the most irritating R41 materials. Materials with a MRD_{50} score of between 2 mg/ml and 10 mg/ml were considered R36 materials, and materials with an MRD_{50} >10 mg/ml were considered to have Not Classified. A significant difficulty with choosing these cut-off values was that there was only one surfactant chemical and no surfactant formulations that carried an R36 classification. Thus it is difficult to have high confidence in the 10 mg/ml cut-off value for the Not Classified classification.

An analysis of the performance statistics that result from using this prediction model are shown in Table 6.1.3.4.1.c (surfactant chemicals) and Table 6.1.3.4.1.d (surfactant containing formulations). These statistics are considerably better than for the non-surfactant materials. There was some under prediction of R41 surfactants or surfactant containing materials (17% and 21%, respectively), but there was considerable over prediction of Not Classified materials (63% for surfactants and 72% for surfactant containing materials).

A further analysis of the ability of the CM to predict either R41 versus the other categories, or EU Not Classified versus the other categories for surfactants and surfactant containing materials is shown in Table 6.1.3.4.f. It can be seen that the positive predictive value (PPV) is much higher for the surfactants and surfactant containing materials (100% for severes vs. the rest [surfactants], 92% severes vs. the rest [surfactant formulations] than it is for non-surfactant chemicals (83% for severes vs. the rest [non-surfactant chemicals].

Because the number of materials that were analyzed either as surfactants or as surfactant-containing materials was still small for each group, we combined these two classes to obtain a data set of 53 test materials of which 50 have unique chemical:concentration combinations and 38 are completely unique chemicals or formulations. For these materials the PPV for severe irritants versus the rest was 95% and

the negative predictive value (NPV) was 84%. For EU non irritants versus the rest, the PPV was 60% and the NPV was 100%.

Table 6.1.3.4.1.a Combined studies – Non-surfactant chemicals and formulations - Contingency table depicting the concordance and predictivity of the CM assay for EU hazard classifications when the cut-off values from Figure 6.1.3.4.1.a are applied. N = 29 non-surfactant materials.

Draize Determined EU Category	EU Category Predicted by CM						
	R41	R36	Not Classified	Total	Concordance	Toxicity Overpredicted	Toxicity Underpredicted
R41	5	5	1	11	45%	NA	55%
R36	1	5	4	10	50%	10%	40%
Not Classified	0	2	6	8	75%	25%	NA
Total	6	12	11	29	55%		
Predictivity	83%	42%	55%				
Category Under predicted	NA	42%	45%				
Category Overpredicted	17%	17%	NA				

Table 6.1.3.4.1.b Combined studies – Non-surfactant chemicals - Contingency table depicting the concordance and predictivity of the CM assay for EU hazard classifications when the cut-off values from Figure 6.1.3.4.1.b are applied. N = 27 non-surfactant chemicals.

Draize Determined EU Category	EU Category Predicted by CM						
	R41	R36	Not Classified	Total	Concordance	Toxicity Overpredicted	Toxicity Underpredicted
R41	5	5	1	11	45%	NA	55%
R36	1	5	3	9	56%	11%	33%
Not Classified	0	1	6	7	86%	14%	NA
Total	6	11	10	27	59%		
Predictivity	83%	45%	60%				
Category Under predicted	NA	45%	40%				
Category Overpredicted	17%	9%	NA				

Table 6.1.3.4.1.c Combined studies – Surfactant chemicals - Contingency table depicting the concordance and predictivity of the CM assay for EU hazard classifications when the cut-off values from Figure 6.1.3.4.1.c are applied. N = 21 surfactant chemicals.

Draize Determined EU Category	EU Category Predicted by CM						
	R41	R36	Not Classified	Total	Concordance	Toxicity Overpredicted	Toxicity Underpredicted
R41	10	2	0	12	83%	NA	17%
R36	0	1	0	1	100%	0%	0%
Not Classified	0	5	3	8	38%	63%	NA
Total	10	8	3	21	67%		
Predictivity	100%	13%	100%				
Category Under predicted	NA	25%	0%				
Category Overpredicted	0%	63%	NA				

Table 6.1.3.4.1.d Combined studies – Surfactant containing formulations - Contingency table depicting the concordance and predictivity of the CM assay for EU hazard classifications when the cut-off values from Figure 6.1.3.4.1.d are applied. N = 32 surfactant formulations.

Draize Determined EU Category	EU Category Predicted by CM						
	R41	R36	Not Classified	Total	Concordance	Toxicity Overpredicted	Toxicity Underpredicted
R41	11	3	0	14	79%	NA	21%
R36	0	0	0	0	NA	NA	NA
Not Classified	1	12	5	18	28%	72%	NA
Total	12	15	5	32	50%		
Predictivity	92%	0%	100%				
Category Under predicted	NA	20%	0%				
Category Overpredicted	8%	80%	NA				

Table 6.1.3.4.1.e Combined studies – Surfactant Chemicals and Formulations - Contingency table depicting the concordance and predictivity of the CM assay for EU hazard classifications when the cut-off values from Figure 6.1.3.4.1.e are applied. N = 53 surfactant materials.

Draize Determined EU Category	EU Category Predicted by CM						
	R41	R36	Not Classified	Total	Concordance	Toxicity Overpredicted	Toxicity Underpredicted
R41	21	5	0	26	80.8%	NA	19.2%
R36	0	1	0	1	100.0%	0%	0%
Not Classified	1	17	8	26	30.8%	69.2%	NA
Total	22	23	8	53	56.6%		
Predictivity	95.5%	4.3%	100.0%				
Category Under predicted	NA	21.7%	0%				
Category Overpredicted	4.5%	73.9%	NA				

6.1.3.4.2 Analysis of the combined CTFA, EC/HO, and COLIPA data with GHS category

Possible prediction models for the GHS hazard classification were determined similarly to that described for the EU classification system described in Section 6.1.3.4.1 by setting cut-off values empirically in a conservative manner that kept under predictions to a minimum. For the combined non-surfactant chemicals and formulations, and non-surfactant chemicals (Figures 6.1.3.4.2.a and 6.1.3.4.2.b), this seemed to be done by cut-off values at 80 mg/ml and 3 mg/ml such that GHS 1 predictions would be made for materials with MRD_{50} values ≤ 3.0 mg/ml, GHS 2A predictions would be made for materials where their MRD_{50} was between 3 and 10 mg/ml (it was not possible to discriminate between 2A and 2B categories) and No Label for materials with MRD_{50} ≥ 80 mg/ml. Cut-off values are represented in the figures by horizontal lines. Some data points which overlapped have been displaced horizontally along the x-axis so that they are more

easily visible. The predictive capacity for this prediction model for non-surfactant chemicals (there were only two non-surfactant formulations) is shown in Table 6.1.3.4.2.b. It can be seen from both the Table and the Figure 6.1.3.4.2.b that there is a high level of under prediction of both GHS 1 (45%) and GHS 2A (60%) categories.

A similar approach was taken for the surfactants and surfactant containing formulations. Figures 6.1.3.4.2.c and 6.1.2.4.2.d show that for these materials a slightly lower cut-off (2 mg/ml) was selected to identify the most irritating GHS 1 materials. Materials with a MRD_{50} score of between 2 mg/ml and 10 mg/ml were considered category 2A materials, and materials with an MRD_{50} >10 mg/ml were considered to have No Label. One main difficulty with choosing these cut-off values was that there were only three surfactant chemicals and two surfactant formulations that carried either a 2A or 2B classification. Thus it is difficult to have high confidence in the 10 mg/ml cut-off value for the No Label classification.

An analysis of the performance statistics that result from using this prediction model are shown in Table 6.1.3.4.2.c (surfactant chemicals) and Table 6.1.3.4.2.d (surfactant containing formulations). These statistics are considerably better than for the non-surfactant materials. There was very little under prediction of GHS 1 surfactants or surfactant containing materials (9% and 8%, respectively), but there was considerable over prediction of No Label materials (57% for surfactants and 73% for surfactant containing materials). The PPV for the extremes of the irritation categories were very good for both surfactants and surfactant containing formulations. For surfactants, the predictivity was 100% for GHS 1's and No Label. For surfactant containing formulations, the predictivity was 92% for GHS 1's and 100% for No Label.

For the combination of surfactants and surfactant containing materials the predictivity was 95.5% for GHS 1's (22/22) and 100% for No Label (8/8).

A further analysis of the ability of the CM to predict either GHS 1 versus the other categories, or GHS No Label versus the other categories is shown in Table 6.1.3.4.g. It can be seen that the PPV is high for the non-surfactant chemicals, surfactants and surfactant containing materials (100% for severes vs. the rest [non-surfactant chemicals], 100% for severes vs. the rest [surfactants] and 92% severes vs. the rest [surfactant formulations]. However, the NPV for the non-surfactants is only 76% while it is 91% for the surfactant chemicals and 95% for the surfactant formulations.

Because the number of materials that were analyzed either as surfactants or as surfactant-containing materials was still small for each group, we combined these two classes to obtain a data set of 53 test materials of which 50 have unique chemical:concentration combinations and 38 are completely unique chemicals or formulations. For these materials the PPV for severe irritants versus the rest was 95% and the NPV was 94%. For GHS non irritants versus the rest, the PPV was 62% and the NPV was 100%.

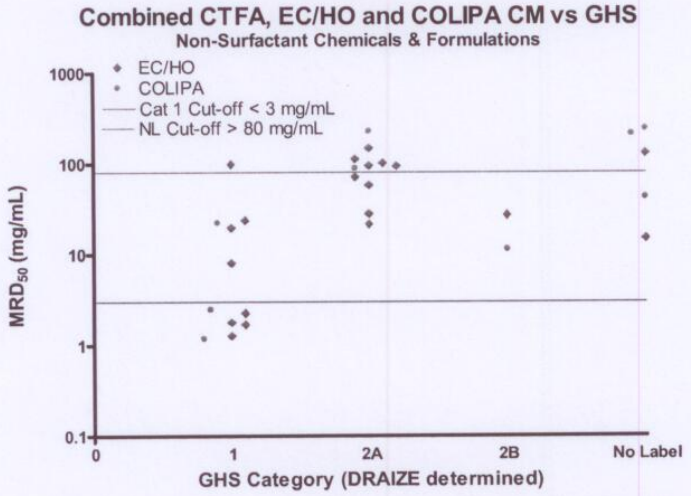


Figure 6.1.3.4.2.a Combined data for all non-surfactant containing chemicals and formulations from the CTFA, EC/HO and COLIPA studies graphed against GHS hazard classifications. Proposed MRD_{50} cut-off values of >80 mg/ml to define No Label materials and <3 mg/ml to define Category 1 materials are shown. There are 29 total materials; 23 unique chemical:concentration combinations, and 21 totally unique chemicals.

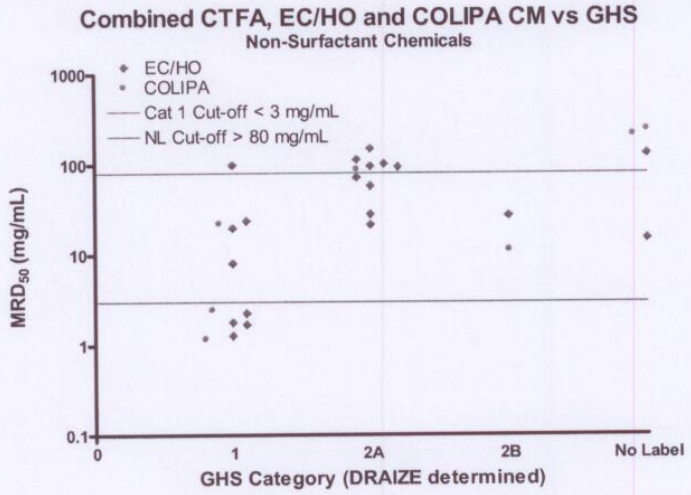


Figure 6.1.3.4.2.b Combined data for non-surfactant containing chemicals from the CTFA, EC/HO and COLIPA studies graphed against GHS hazard classifications. Proposed MRD_{50} cut-off values of >80 mg/ml to define No Label materials and <3 mg/ml to define Category 1 materials are shown. There are 27 total materials; 21 unique chemical:concentration combinations, and 19 totally unique chemicals.

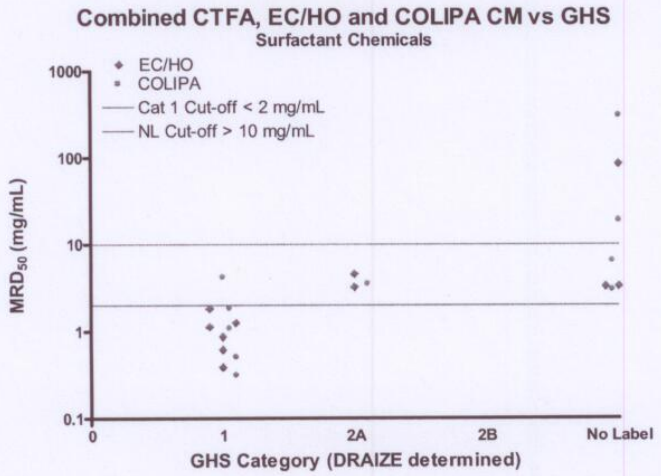


Figure 6.1.3.4.2.c Combined data for surfactant containing chemicals from the CTFA, EC/HO and COLIPA studies graphed against GHS hazard classifications. Proposed MRD_{50} cut-off values of >10 mg/ml to define No Label materials and <2 mg/ml to define Category 1 materials are shown. There are 21 total materials; 13 unique chemical:concentration combinations, and 6 totally unique chemicals.

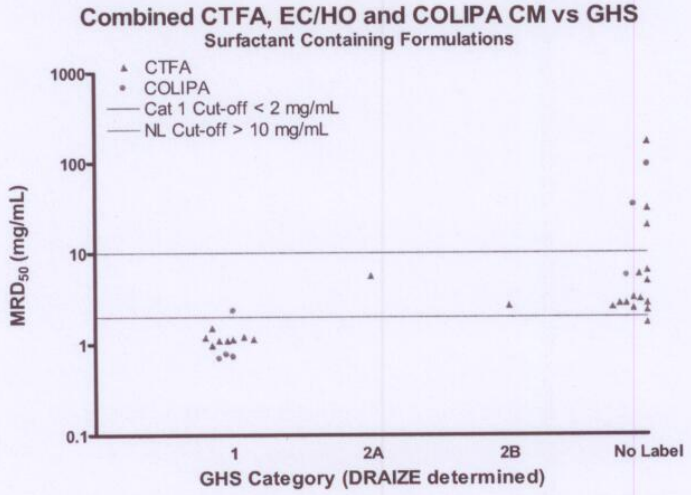


Figure 6.1.3.4.2.d Combined data for surfactant containing formulations from the CTFA, EC/HO and COLIPA studies graphed against GHS hazard classifications. Proposed MRD_{50} cut-off values of >10 mg/ml to define No Label materials and <2 mg/ml to define Category 1 materials are shown. There are 32 totally unique chemicals. The assumption is that the six reformulated test materials for the COLIPA study are different from similar test materials used in the CTFA study.

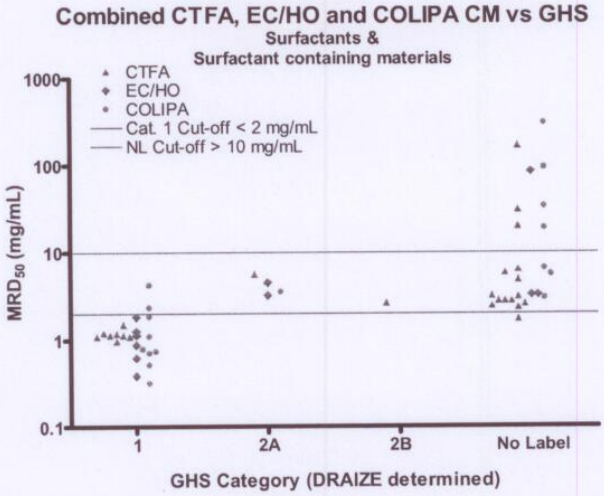


Figure 6.1.3.4.2.e Combined data from the CTFA, EC/HO and COLIPA studies graphed against GHS hazard classifications. Proposed MRD_{50} cut-off values of >10 mg/ml to define No Label materials and <2 mg/ml to define category 1 materials are shown. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude. There are 53 total materials; 45 unique chemical:concentration combinations, and 38 totally unique chemicals/formulations.

Table 6.1.3.4.2.a Combined studies – Non-surfactant chemicals and formulations - Contingency table depicting the concordance and predictivity of the CM assay for GHS hazard classifications when the cut-off values from Figure 6.1.3.4.2.a are applied. N = 29 non-surfactant materials.

Draize Determined GHS Category	GHS Category Predicted By CM						
	1	2A	2B	No Label	Total	Concordance	Toxicity Overpredicted	Toxicity Underpredicted
1	6	4		1	11	55%	NA	45%
2A	0	4		7	11	36%	0%	64%
2B	0	2		0	2	100%	0%	0%
No Label	0	2		3	5	60%	40%	NA
Total	6	12		11	29	52%		
Predictivity	100%	50%		27%				
Category Under predicted	NA	33%		73%				
Category Overpredicted	0%	17%		NA				

Table 6.1.3.4.2.b Combined studies – Non-surfactant chemicals - Contingency table depicting the concordance and predictivity of the CM assay for GHS hazard classifications when the cut-off values from Figure 6.1.3.4.2.b are applied. N = 27 non-surfactant chemicals.

Draize Determined GHS Category	GHS Category Predicted By CM							
	1	2A	2B	No Label	Total	Concordance	Toxicity Overpredicted	Toxicity Underpredicted
1	6	4		1	11	55%	NA	45%
2A	0	4		6	10	40%	0%	60%
2B	0	2		0	2	100%	0%	0%
No Label	0	1		3	4	75%	25%	NA
Total	6	11		10	27	56%		
Predictivity	100%	55%		30%				
Category Under predicted	NA	36%		70%				
Category Overpredicted	0%	9%		NA				

Table 6.1.3.4.2.c Combined studies – Surfactant chemicals - Contingency table depicting the concordance and predictivity of the CM assay for GHS hazard classifications when the cut-off values from Figure 6.1.3.4.2.c are applied. N = 21 surfactant chemicals.

Draize Determined GHS Category	GHS Category Predicted By CM							
	1	2A	2B	No Label	Total	Concordance	Toxicity Overpredicted	Toxicity Underpredicted
1	10	1		0	11	91%	NA	9%
2A	0	3		0	3	100%	0%	0%
2B	0	0		0	0	NA	NA	NA
No Label	0	4		3	7	43%	57%	NA
Total	10	8		3	21	76%		
Predictivity	100%	38%		100%				
Category Under predicted	NA	13%		0%				
Category Overpredicted	0%	50%		NA				

Table 6.1.3.4.2.d Combined studies – Surfactant containing formulations - Contingency table depicting the concordance and predictivity of the CM assay for GHS hazard classifications when the cut-off values from Figure 6.1.3.4.2.d are applied. N = 32 surfactant formulations.

Draize Determined GHS Category	GHS Category Predicted By CM							
	1	2A	2B	No Label	Total	Concordance	Toxicity Overpredicted	Toxicity Underpredicted
1	11	1		0	12	92%	NA	8%
2A	0	1		0	1	100%	0%	0%
2B	0	1		0	1	100%	0%	0%
No Label	1	12		5	18	28%	73%	NA
Total	12	15		5	32	56%		
Predictivity	92%	13%		100%				
Category Under predicted	NA	7%		0%				
Category Overpredicted	8%	80%		NA				

Table 6.1.3.4.2.e Combined studies – Surfactants and surfactant containing materials - Contingency table depicting the concordance and predictivity of the CM assay for GHS hazard classifications when the cut-off values from Figure 6.1.3.4.2.e are applied. N = 53 surfactant materials.

Draize Determined GHS Category	GHS Category Predicted By CM							
	1	2A	2B	No Label	Total	Concordance	Toxicity Overpredicted	Toxicity Underpredicted
1	21	2		0	23	91.3%	NA	8.7%
2A	0	4		0	4	100.0%	0%	0%
2B	0	1		0	1	100.0%	0%	0%
No Label	1	16		8	25	32.0%	68.0%	NA
Total	22	23		8	53	64.2%		
Predictivity	95.5%	21.7%		100.0%				
Category Under predicted	NA	8.7%		0%				
Category Overpredicted	4.5%	69.6%		NA				

6.1.3.4.3 Analysis of the combined CTFA, EC/HO, and COLIPA data with EPA category

Possible prediction models for the EPA hazard classification were determined similarly to that described for the EU classification system described in Section 6.1.3.4.1 by setting cut-off values empirically in a conservative manner that kept under predictions to a minimum. For the combined non-surfactant chemicals and formulations, and non-surfactant chemicals (Figures 6.1.3.4.3.a and 6.1.3.4.3.b), this seemed to be done by cut-off values at 80 mg/ml and 3 mg/ml such that EPA I predictions would be made for materials with MRD_{50} values ≤ 3.0 mg/ml, EPA II predictions would be made for materials where their MRD_{50} was between 3 and 10 mg/ml (it was not possible to discriminate between EPA II and EPA III categories) and EPA IV for materials with MRD_{50} ≥ 80 mg/ml. Cut-off values are represented in the figures by horizontal lines. Some data points which overlapped have been displaced horizontally along the x-axis so that they are more easily visible. The predictive capacity for this prediction model for non-surfactant chemicals (there were only two non-surfactant formulations) is shown in Table 6.1.3.4.3.b. It can be seen from both the Table and the Figure 6.1.3.4.3.b that there is a high level of under prediction of both EPA I (43%) and EPA II (60%) categories.

A similar approach was taken for the surfactants and surfactant containing formulations. Figures 6.1.3.4.3.c and 6.1.2.4.3.d show that for these materials a slightly lower cut-off (2 mg/ml) was selected to identify the most irritating EPA I materials. Materials with a MRD_{50} score of between 2 mg/ml and 80 mg/ml were considered category II materials, and materials with an MRD_{50} >80 mg/ml were considered to be EPA IV. One main difficulty with choosing these cut-off values was that there was only one surfactant chemical that carried an EPA IV classification.

Analyses of the performance statistics that result from using this prediction model are shown in Table 6.1.3.4.3.c (surfactant chemicals) and Table 6.1.3.4.3.d (surfactant containing formulations). These statistics are better than for the non-surfactant materials.

There was some under prediction of EPA I surfactants or surfactant containing materials (22% and 21%, respectively), and also some over prediction of EPA IV materials (0% for surfactants [but only one material was in that category] and 60% for surfactant containing materials). The positive predictive values for the extremes of the irritation categories were in general good for both surfactants and surfactant containing formulations. For surfactants, the predictivity was 78% for EPA I's and 50% for EPA IV's. For surfactant containing formulations, the predictivity was 92% for EPA I's and 100% for EPA IV's.

For the combination of surfactants and surfactant containing materials the predictivity was 85.7% for EPA I's (18/21) and 75% for EPA IV's (3/4).

A further analysis of the ability of the CM to predict either EPA I versus the other categories or EPA IV versus the other categories is shown in Table 6.1.3.4.h. It can be seen that the PPV is moderate for the non-surfactant chemicals, surfactants and surfactant containing materials (80% for severes vs. the rest [non-surfactant chemicals], 78% for severes vs. the rest [surfactants] and 92% severes vs. the rest [surfactant formulations]. The NPV for the non-surfactants is 83%, while it is 82% for the surfactant chemicals and 85% for the surfactant formulations.

Because the number of materials that were analyzed either as surfactants or as surfactant-containing materials was still small for each group, we combined these two classes to obtain a data set of 52 test materials of which 49 have unique chemical:concentration combinations and 38 are completely unique chemicals or formulations. For these materials the PPV for severe irritants versus the rest was 86% and the NPV was 84%. For EU non irritants versus the rest, the PPV was 94% and the NPV was 75%.

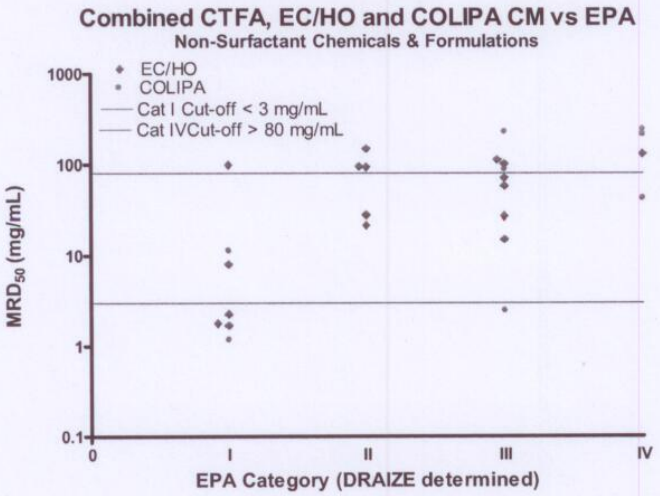


Figure 6.1.3.4.3.a Combined data for all non-surfactant chemicals and formulations from the CTFA, EC/HO and COLIPA studies graphed against EPA hazard classifications. Proposed MRD_{50} cut-off values of >80 mg/ml to define category IV materials and <3 mg/ml to define category I materials are shown. There are 25 total materials; 20 unique chemical:concentration combinations, and 18 totally unique chemicals.

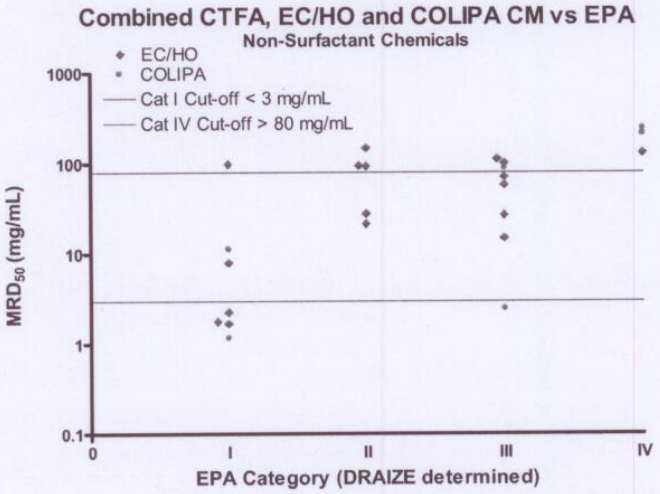


Figure 6.1.3.4.3.b Combined data for non-surfactant chemicals from the CTFA, EC/HO and COLIPA studies graphed against EPA hazard classifications. Proposed MRD_{50} cut-off values of >80 mg/ml to define category IV materials and <3 mg/ml to define category I materials are shown. There are 23 total materials; 18 unique chemical:concentration combinations, and 16 totally unique chemicals.

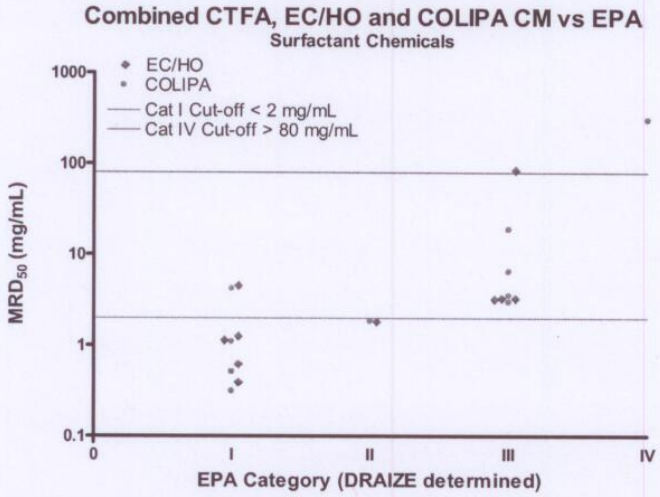


Figure 6.1.3.4.3.c Combined data for surfactant chemicals from the CTFA, EC/HO and COLIPA studies graphed against EPA hazard classifications. Proposed MRD_{50} cut-off values of >80 mg/ml to define category IV materials and <2 mg/ml to define category I materials are shown. There are 20 total materials; 12 unique chemical:concentration combinations, and 6 totally unique chemicals.

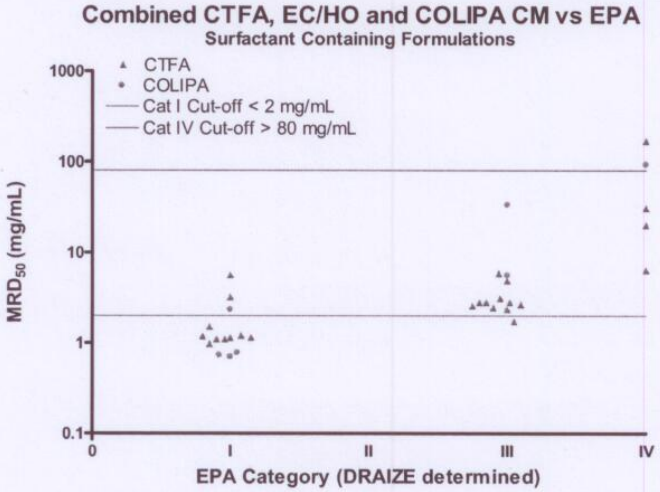


Figure 6.1.3.4.3.d Combined data for surfactant formulations from the CTFA, EC/HO and COLIPA studies graphed against EPA hazard classifications. Proposed MRD_{50} cut-off values of >80 mg/ml to define category IV materials and <2 mg/ml to define category I materials are shown. There are 32 totally unique chemicals. The assumption is that the six reformulated test materials for the COLIPA study are different from similar test materials used in the CTFA study.

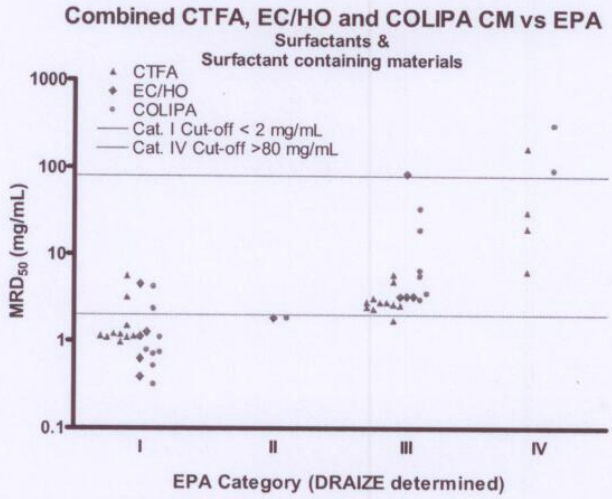


Figure 6.1.3.4.3.e Combined data for all surfactants and surfactant containing materials from the CTFA, EC/HO and COLIPA studies graphed against EPA hazard classifications. Proposed MRD_{50} cut-off values of >80 mg/ml to define Category IV materials and <2 mg/ml to define Category I materials are shown. In some cases data points have been slightly offset along the X-axis in order to clearly separate them from data of similar magnitude. There are 52 total materials; 44 unique chemical:concentration combinations, and 38 totally unique chemicals/formulations.

Table 6.1.3.4.3.a Combined studies – Non-surfactant chemicals and formulations - Contingency table depicting the concordance and predictivity of the CM assay for EPA hazard classifications when the cut-off values from Figure 6.1.3.4.3.a are applied. N = 25 non-surfactant materials.

Draize Determined EPA Category	EPA Category Predicted By CM						
	I	II	III	IV	Total	Concordance	Toxicity Overpredicted	Toxicity Underpredicted
I	4	2		1	7	57%	NA	43%
II	0	2		3	5	40%	0%	60%
III	1	4		4	9	44%	11%	44%
IV	0	1		3	4	75%	25%	NA
Total	5	9		11	25	52%		
Predictivity	80%	67%		27%				
Category Underpredicted	NA	22%		73%				
Category Overpredicted	20%	11%		NA				

Table 6.1.3.4.3.b Combined studies – Non-surfactant chemicals - Contingency table depicting the concordance and predictivity of the CM assay for EPA hazard classifications when the cut-off values from Figure 6.1.3.4.3.b are applied. N = 23 non-surfactant chemicals.

Draize Determined EPA Category	EPA Category Predicted By CM						
	I	II	III	IV	Total	Concordance	Toxicity Overpredicted	Toxicity Underpredicted
I	4	2		1	7	57%	NA	43%
II	0	2		3	5	40%	0%	60%
III	1	4		3	8	50%	13%	37%
IV	0	0		3	3	100%	0%	NA
Total	5	8		10	23	57%		
Predictivity	80%	75%		30%				
Category Underpredicted	NA	25%		70%				
Category Overpredicted	20%	0%		NA				

Table 6.1.3.4.3.c Combined studies – Surfactant chemicals - Contingency table depicting the concordance and predictivity of the CM assay for EPA hazard classifications when the cut-off values from Figure 6.1.3.4.3.c are applied. N = 20 surfactant chemicals.

Draize Determined EPA Category	EPA Category Predicted By CM						
	I	II	III	IV	Total	Concordance	Toxicity Overpredicted	Toxicity Underpredicted
I	7	2		0	9	78%	NA	22%
II	2	0		0	2	0%	100%	0%
III	0	7		1	8	88%	0%	13%
IV	0	0		1	1	100%	0%	NA
Total	9	9		2	20	75%		
Predictivity	78%	78%		50%				
Category Underpredicted	NA	22%		50%				
Category Overpredicted	22%	0%		NA				

Table 6.1.3.4.3.d Combined studies – Surfactant containing formulations - Contingency table depicting the concordance and predictivity of the CM assay for EPA hazard classifications when the cut-off values from Figure 6.1.3.4.3.d are applied. N = 32 surfactant formulations.

Draize Determined EPA Category	EPA Category Predicted By CM						
	I	II	III	IV	Total	Concordance	Toxicity Overpredicted	Toxicity Underpredicted
I	11	3		0	14	79%	NA	21%
II	0	0		0	0	NA	NA	NA
III	1	12		0	13	92%	8%	0%
IV	0	3		2	5	40%	60%	NA
Total	12	18		2	32	78%		
Predictivity	92%	67%		100%				
Category Underpredicted	NA	17%		0%				
Category Overpredicted	8%	17%		NA				

Table 6.1.3.4.3.e Combined studies - Surfactant Chemicals and Formulations - Contingency table depicting the concordance and predictivity of the CM assay for EPA hazard classifications when the cut-off values from Figure 6.1.3.4.3.e are applied. N = 52 surfactant materials.

Draize Determined EPA Category	EPA Category Predicted By CM						
	I	II	III	IV	Total	Concordance	Toxicity Overpredicted	Toxicity Underpredicted
I	18	5		0	23	78.3%	NA	21.7%
II	2	0		0	2	0.0%	100.0%	0%
III	1	19		1	21	90.5%	4.8%	4.8%
IV	0	3		3	6	50.0%	50.0%	NA
Total	21	27		4	52	76.9%		
Predictivity	85.7%	70.4%		75.0%				
Category Underpredicted	NA	18.5%		25.0%				
Category Overpredicted	14.3%	11.1%		NA				

An analysis of the ability of the CM assay to test surfactants and surfactant-based materials and separate severe irritants from "the rest", and non-irritants from "the rest" is shown in Table 6.1.3.4.m. Using the larger data set of materials combined from the HO/EC, CTFA Phase III and COLIPA data sets, the CM assay seemed to perform reasonably well when separating EU and GHS severe irritants from "the rest" (both had positive predictivities of 100% and negative predictivities of 78%). However, this analysis is confounded some what by the uncertainty of how many truly "unique" materials are represented in overlapping data set of 53 (or 52 EPA) materials.

Table 6.1.3.4.f Combined studies - Concordance for severe irritants versus the rest and non-irritants versus the rest for the combined studies. Cut-off values from Figures 6.1.3.4.1.a – 6.1.3.4.1.e were applied. N = 29 non-surfactant materials, 27 non-surfactant chemicals, 21 surfactant chemicals, 32 surfactant formulations, and 53 surfactant materials.

		Vs Rest													
		Concordance		Sensitivity		Specificity		Positive Predictivity		Negative Predictivity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.
Non-Surfactant Chemicals and Formulations	EU Severe irritants	76%	22/29	45%	5/11	94%	17/18	83%	5/6	74%	17/23	6%	1/18	55%	6/11
	EU non-irritants	76%	22/29	76%	16/21	75%	6/8	89%	16/18	55%	6/11	25%	2/8	24%	5/21
Non-Surfactant Chemicals	EU Severe irritants	74%	20/27	45%	5/11	94%	15/16	83%	5/6	71%	15/21	6%	1/16	55%	6/11
	EU non-irritants	81%	22/27	80%	16/20	86%	6/7	94%	16/17	60%	6/10	14%	1/7	20%	4/20
Surfactant Chemicals	EU Severe irritants	90%	19/21	83%	10/12	100%	9/9	100%	10/10	82%	9/11	0%	0/9	17%	2/12
	EU non-irritants	76%	16/21	100%	13/13	38%	3/8	72%	13/18	100%	3/3	63%	5/8	0%	0/13
Surfactant Formulations	EU Severe irritants	88%	28/32	79%	11/14	94%	17/18	92%	11/12	85%	17/20	6%	1/18	21%	3/14
	EU non-irritants	59%	19/32	100%	14/14	28%	5/18	52%	14/27	100%	5/5	72%	13/18	0%	0/14
Surfactant Chemicals and Formulations	EU Severe irritants	89%	47/53	81%	21/26	96%	26/27	95%	21/22	84%	26/31	4%	1/27	19%	5/26
	EU non-irritants	66%	35/53	100%	27/27	31%	8/26	60%	27/45	100%	8/8	69%	18/26	0%	0/27

Table 6.1.3.4.g Combined studies - Concordance for severe irritants versus the rest and non-irritants versus the rest for the combined studies. Cut-off values from Figures 6.1.3.4.2.a – 6.1.3.4.2.e were applied. N = 29 non-surfactant materials, 27 non-surfactant chemicals, 21 surfactant chemicals, 32 surfactant formulations, and 53 surfactant materials.

		Vs Rest													
		Concordance		Sensitivity		Specificity		Positive Predictivity		Negative Predictivity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.
Non-Surfactant Chemicals and Formulations	GHS Severe irritants	83%	24/29	55%	6/11	100%	18/18	100%	6/6	78%	18/23	0%	0/18	45%	5/11
	GHS non-irritants	66%	19/29	67%	16/24	60%	3/5	89%	16/18	27%	3/11	40%	2/5	33%	8/24
Non-Surfactant Chemicals	GHS Severe irritants	81%	22/27	55%	6/11	100%	16/16	100%	6/6	76%	16/21	0%	0/16	45%	5/11
	GHS non-irritants	70%	19/27	70%	16/23	75%	3/4	94%	16/17	30%	3/10	25%	1/4	30%	7/23
Surfactant Chemicals	GHS Severe irritants	95%	20/21	91%	10/11	100%	10/10	100%	10/10	91%	10/11	0%	0/10	9%	1/11
	GHS non-irritants	81%	17/21	100%	14/14	43%	3/7	78%	14/18	100%	3/3	57%	4/7	0%	0/14
Surfactant Formulations	GHS Severe irritants	94%	30/32	92%	11/12	95%	19/20	92%	11/12	95%	19/20	5%	1/20	8%	1/12
	GHS non-irritants	59%	19/32	100%	14/14	28%	5/18	52%	14/27	100%	5/5	72%	13/18	0%	0/14
Surfactant Chemicals and Formulations	GHS Severe irritants	94%	50/53	91%	21/23	97%	29/30	95%	21/22	94%	29/31	3%	1/30	9%	2/23
	GHS non-irritants	68%	36/53	100%	28/28	32%	8/25	62%	28/45	100%	8/8	68%	17/25	0%	0/28

Table 6.1.3.4.h Combined studies - Concordance for severe irritants versus the rest and non-irritants versus the rest for the combined studies. Cut-off values from Figures 6.1.3.4.3.a – 6.1.3.4.3.e were applied. N = 25 non-surfactant materials, 23 non-surfactant chemicals, 20 surfactant chemicals, 32 surfactant formulations, and 52 surfactant materials.

		Vs Rest													
		Concordance		Sensitivity		Specificity		Positive Predictivity		Negative Predictivity		False Positive Rate		False Negative Rate	
		%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.
Non-Surfactant Chemicals and Formulations	EPA Severe irritants	84%	21/25	57%	4/7	94%	17/18	80%	4/5	85%	17/20	6%	1/18	43%	3/7
	EPA non-irritants	64%	16/25	62%	13/21	75%	3/4	93%	13/14	27%	3/11	25%	1/4	38%	8/21
Non-Surfactant Chemicals	EPA Severe irritants	83%	19/23	57%	4/7	94%	15/16	80%	4/5	83%	15/18	6%	1/16	43%	3/7
	EPA non-irritants	70%	16/23	65%	13/20	100%	3/3	100%	13/13	30%	3/10	0%	0/3	35%	7/20
Surfactant Chemicals	EPA Severe irritants	80%	16/20	78%	7/9	82%	9/11	78%	7/9	82%	9/11	18%	2/11	22%	2/9
	EPA non-irritants	95%	19/20	95%	18/19	100%	1/1	100%	18/18	50%	1/2	0%	0/1	5%	1/19
Surfactant Formulations	EPA Severe irritants	88%	28/32	79%	11/14	94%	17/18	92%	11/12	85%	17/20	6%	1/18	21%	3/14
	EPA non-irritants	91%	29/32	100%	27/27	40%	2/5	90%	27/30	100%	2/2	60%	3/5	0%	0/27
Surfactant Chemicals and Formulations	EPA Severe irritants	85%	44/52	78%	18/23	90%	26/29	86%	18/21	84%	26/31	10%	3/29	22%	5/23
	EPA non-irritants	92%	48/52	98%	45/46	50%	3/6	94%	45/48	75%	3/4	50%	3/6	2%	1/46

6.1.3.5 Analysis of Company # 1 unpublished data

The reference data for the Company # 1 studies (Annex K) are all from LVET studies. We were informed by Company # 1 that these data were gathered over a number of years from studies carried out at various animal facilities. Some of the studies were done with full GLP compliance and some were not; however, there is no indication in the data received from Company # 1 which did have GLP compliance. The reference chemicals are mainly raw surfactants and surfactant-containing formulations. There were 76 materials which had unique CM values although 80 materials are listed in Table 6.1.3.5.a. The four additional listings represent a second animal test for 4 separate materials. The results of the second animal test for these materials were listed to give an indication of the variability of the animal test. It should be noted that in each of the four sets of duplicate animal tests at least one of the EU, GHS or EPA categories differed in one test from what was found in a presumably duplicate test. The variability of the LVET has been shown to be similar to that of the traditional Draize test (Cormier, Parker et al. 1996).

The 76 materials which make of this data set are highly biased towards mildness; the vast majority of the tests (61 out of 80 tests; 76%) are Not Classified when using the EU system, or have No Category (39 out of 80 tests; 49%) when using the GHS system. However, when using the EPA scoring system a much smaller fraction (13 out of 80 tests; 16%) are classified as Category IV materials. Specifically under the EU system 61 are Not Classified, 10 (12%) are R36 and 9 (11%) are R41. Under the GHS system 39 have No Category, 17 (21%) are Category 2B, 16 (20%) are Category 2A, and 8 (10%) are Category 1. Under the EPA system 13 are Category IV, 47 (59%) are Category III, 11 (14%) are Category II and 9 (11%) are Category I.

The *in vitro* data come from primarily two sources – internal Company # 1 laboratories and Company # 4 (later the Company # 3). *In vitro* data were generated by both the SM and CM. For the purposes of this analysis, all SM MRD_{50} values have been transformed to equivalent CM MRD_{50} values by an algorithm described earlier in this BRD in section 2.2.1.1. Raw data (including the original SM MRD_{50} values) for these studies can be found in Annex F.

Because the Company # 1 materials were being tested for commercial use when the data were generated, the identities of the materials will not be made publicly available for this BRD. However, the materials have been characterized by Company # 1 staff with respect to the type of ingredient being tested or to the primary components of the formulation. Only those materials which are described as surfactants or as formulations which are surfactant based are analyzed in the following section.

Table 6.1.3.5.a Summary of Company # 1 study. N = 76 materials.

In Vivo Eye Irritation Classifications – Company 1										
BRD chemical number	Substance	CASRN[1]	Concentration Tested	Purity (%)	n. of animals	*In Vivo* EU[2,3]	*In Vivo* GHS[4,5]	*In Vivo* EPA[6,7]	ECETOC MMAS Score[8]	MRD_{50} (mg/mL)
1001					6	Not classified	No Category	Category III		0.435
1002					6	Not classified	No Category	Category III		0.535
1003					6	Not classified	Category 2A	Category II		0.44
1004					3	Not classified	Category 2B	Category III		0.421
1005					3	Not classified	Category 2A	Category II		0.411
1006					3	Not classified	No Category	Category III		0.443
1007					3	Not classified	Category 2B	Category III		0.428
1008					3	Not classified	Category 2B	Category III		0.272
1009					6	Not classified	No Category	Category III		0.465
1010					3	R41	Category 1	Category I		0.456
1011					3	R41	Category 1	Category I		0.44
1012					3	Not classified	Category 2A	Category II		0.415
1013					3	Not classified	Category 2B	Category III		0.426
1014					3	Not classified	No Category	Category III		0.444
1015					3	Not classified	No Category	Category III		0.412
1016					3	Not classified	Category 2B	Category III		0.272
1017					3	Not classified	Category 2B	Category III		0.432
1018					3	R36	Category 2B	Category III		0.465
1019					3	R41	Category 1	Category I		0.276
1020					3	R41	Category 1	Category I		0.296
1021					6	Not classified	No Category	Category III		0.19
1022					6	R41	Category 2A	Category I		0.51
1023	**Same as 1022; 2nd animal study***				6	R36	Category 2A	Category III		
1024					3	R41	Category 1	Category I		0.2
1025					6	Not classified	No Category	Category III		0.829
1026					6	Not classified	Category 2B	Category III		0.434
1027					6	Not classified	No Category	Category III		0.44
1028					3	Not classified	Category 2A	Category III		0.46
1029					3	R36	Category 2B	Category III		0.45
1030					3	Not classified	Category 2B	Category III		0.6
1031					3	R36	Category 2B	Category III		0.5
1032					3	R36	Category 2A	Category III		0.96
1033	**Same as 1032; 2nd animal study***				3	Not classified	Category 2B	Category III		
1034					3	Not classified	No Category	Category III		0.67
1035					3	Not classified	No Category	Category IV		63.9
1036					3	Not classified	No Category	Category III		0.79

1039		3	R41	Category 1	Category I	0.26
1040		3	R36	Category 2A	Category II	0.76
1041		3	R36	Category 2A	Category II	0.22
1043		6	Not classified	Category 2A	Category II	0.407
1044		6	Not classified	Category 2A	Category II	0.428
1045		6	Not classified	Category 2A	Category III	0.344
1046		3	R36	Category 2A	Category II	0.264
1047		3	Not classified	No Category	Category III	0.286
1051		3	Not classified	Category 2B	Category III	7.103
1052		3	Not classified	No Category	Category III	1.354
1053		3	Not classified	Category 2B	Category III	0.0808
1054		3	Not classified	Category 2B	Category III	0.0773
1055		3	R36	Category 2A	Category II	0.638
1056		3	R36	Category 2A	Category II	0.817
1057	**Same as 1056; 2nd animal study***	3	R41	Category 1	Category I	
1058		3	Not classified	Category 2A	Category II	0.81
1059		3	Not classified	No Category	Category IV	0.787
1060		3	Not classified	No Category	Category III	0.9
1061		3	Not classified	No Category	Category III	26.733
1062		3	Not classified	No Category	Category IV	46.5
1063		3	Not classified	No Category	Category III	43.1
1064		3	Not classified	No Category	Category III	0.501
1065		3	Not classified	No Category	Category IV	300
1066		3	Not classified	No Category	Category III	3.8
1067		3	Not classified	No Category	Category III	2.573
1068		3	Not classified	No Category	Category III	4.308
1069		3	Not classified	No Category	Category III	0.556
1070		3	Not classified	No Category	Category III	1.96
1071		3	Not classified	No Category	Category IV	0.66
1072		3	Not classified	No Category	Category IV	3.718
1074		3	Not classified	No Category	Category III	4.19
1075		6	Not classified	No Category	Category IV	10.96
1076		6	Not classified	No Category	Category III	0.63
1077			R41	Category 1	Category I	0.63
1078		6	Not classified	No Category	Category III	0.49
1079		3	Not classified	Category 2B	Category III	0.71
1080	**Same as 1079; 2nd animal study***	3	Not classified	No Category	Category IV	
1081		3	Not classified	No Category	Category IV	0.72
1082		3	Not classified	No Category	Category IV	2.02
1083		3	Not classified	No Category	Category III	1.43

1084	3	Not classified	No Category	Category IV	3.86
1085	3	Not classified	No Category	Category IV	15.18
1086	6	Not classified	Category 2B	Category III	0.93
1087	3	Not classified	No Category	Category IV	2.49

[1]CASRN=Chemical Abstract Services Registry Number

[2]EU=European Union (EU [2001]).

[3]Risk phrase R41 = risk of serious damage to the eyes; R36 = irritating to the eyes; not classified.

[4]GHS=Globally Harmonized System (UN [2003])

[5]Eye Irritant Category 1 = irreversible effects on the eye/serious damage to the eye; Category 2A = reversible effects on the eye/irritating to the eyes; Category 2B = reversible effects on the eye/mildly irritating to the eyes; No category

[6]EPA=U.S. Environmental Protection Agency (EPA [1996]).

[7]Toxicity Category I for the Primary Eye Irritation Study = Corrosive, or corneal involvement or irritation not reversible within 21 days; Category II = Corneal involvement or irritation clearing in 8-21 days; Category III = Corneal involvement or irritation clearing in 1-7 daysl; Category IV: minimal effects clearing in less than 24 hr

[9]MMAS scores reported in Harbell et al. (1999)

[8]SCNM=Study Crtieria Not Met

[10]n.p.=not provided

* - Most severe animal data used for graphing purposes.

In order to visualize the relationship between the CM MRD_{50} data and the EU, GHS, and EPA hazard classification system, scatter plot graphs were constructed of MRD_{50} versus hazard category. Figure 6.1.3.5.a depicts the EU categories, Figure 6.1.3.5.b depicts the GHS categories, and Figure 6.1.3.5.c depicts the EPA categories. Starting with EU hazard classification, a possible prediction model was determined empirically by trying to balance over predictions and under predictions in a conservative manner that kept under predictions to a minimum. However, the grouping of the data points showed that there was no obvious difference in MRD_{50} values between R36 and R41 materials. Therefore, we focused just on separating the No Label materials from the combination of R41 and R36 materials. A conservative cut-off value seemed to be at MRD_{50} >2 mg/ml for the No Label materials. This is somewhat above the highest R36 value and seems to be a reasonable approach since there are only eight R36 materials in the analysis. Thus an MRD_{50} score >2 mg/ml would be considered to have a No Label category. Materials with values ≤2 mg/ml would be considered to be either R41 or R36 with the default being the most severe R41 category. The exact designation would have to be determined by a second *in vitro* test which was validated to be able to differentiate between R36 and R41 materials. Figure 6.1.3.5.a shows the 76 data points and the cut-off value represented by a horizontal line.

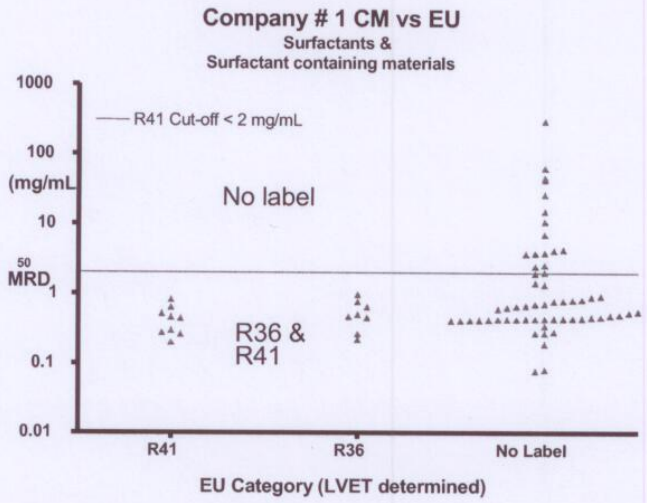


Figure 6.1.3.5.a Results from 76 materials from Company # 1 related to EU classification. For the four materials in Table 6.1.3.5.a which had 2 independent animal tests only the most irritating animal scores were used in this figure.

When the above proposed prediction model (MRD_{50} >2 mg/ml) is applied to the data in Figure 6.1.3.5.a, 16 of 59 (27.1%) of the materials determined to be Not Classified by the animal test would be correctly identified by the CM. This means that 72.9% of the animal designated Not Classified materials would have to be tested in a second level test. None of the materials identified as R41 or R36 by the animal test would be under predicted.

Applying a slightly more aggressive cut-off of MRD_{50} > 1 mg/ml = Not Classified, would raise the number of correctly identified Not Classified materials to 19 out of 59 (32.2%), again with no under prediction of R41 or R36 materials. However, since the 1 mg/ml cut-off is just above the lowest score of the R36 materials, it would seem that more R36 materials should be tested to determine if the range of MRD_{50} values found in this study holds for a larger data set before accepting a cut-off of 1 mg/ml.

An assay of this type could be very useful as a screening assay for products which are very mild. Any products or materials which are below the cut-off might be excluded because of their toxicity, but materials scoring above the cut-off could be reasonably assured to be a non-irritant.

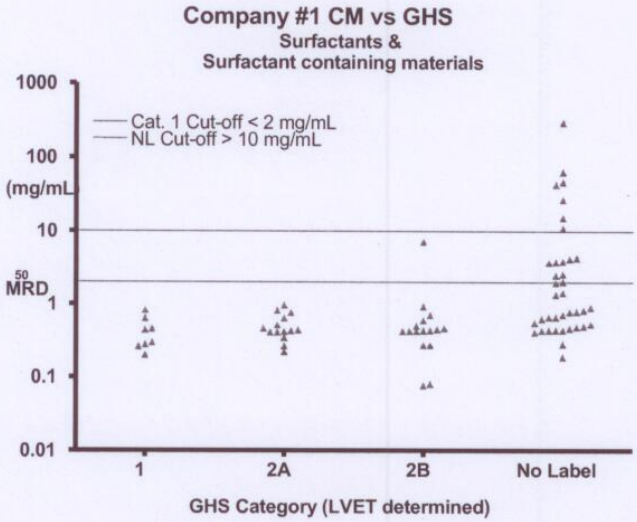


Figure 6.1.3.5.b Results from 76 materials from Company # 1 related to GHS classification. For the four materials in Table 6.1.3.5.b which had 2 independent animal tests only the most irritating animal scores were used in this figure.

As with the EU analysis, the GHS hazard classification analysis began with an attempt to set cut-offs which balanced over predictions and under predictions in a conservative manner that kept under predictions to a minimum. However, the grouping of the data points (Figure 6.1.3.5.b) showed that there was no obvious difference in MRD_{50} values between Category 1, 2A and 2B materials. Again we focused just on separating the No Category materials from the combination of Category 1, 2A and 2B materials. A conservative cut-off value seemed to be at MRD_{50} >10 mg/ml. This is slightly above the highest Category 2B value which seems to be a reasonable approach since there only 16 Category 2B materials in the analysis and it would reasonably assure that no under predictions would occur. Thus an MRD_{50} score >10 mg/ml would be considered to have No Category. Materials with values ≤2 mg/ml would have to be considered to be Category 1 materials unless there was a second *in vitro* test which was validated to successfully categorize them as either 1, 2A, or 2B. Figure 6.1.3.5.b shows the 76 data points and the cut-off values represented by horizontal lines at MRD_{50} = 10 mg/ml and MRD_{50} =2 mg/ml.

An assay of this type could be very useful as a screening assay for products which are very mild. Any products or materials which are below the cut-off might be excluded because of their toxicity, but materials scoring above the cut-off could be reasonably assured to be non-irritant.

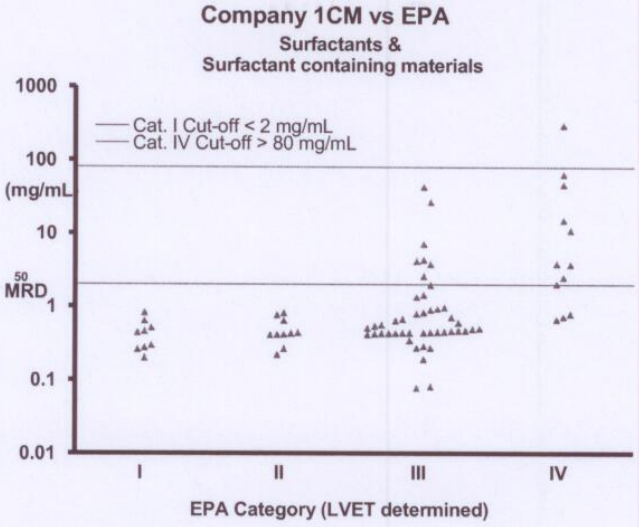


Figure 6.1.3.5.c Results from 76 materials from Company 1 related to EPA classification. For the four materials in Table 6.1.3.5.c which had 2 independent animal tests only the most irritating animal scores were used in this figure.

For the analysis according to the EPA scoring system (Figure 6.1.3.5.c) we again began with an attempt to set cut-offs which balanced over predictions and under predictions in a conservative manner that kept under predictions to a minimum. However, the grouping of the data points (Figure 6.1.3.5.c) showed that there was no obvious difference in MRD_{50} values between Category I, II and a majority of the Category III materials. Therefore, we focused just on separating the highly irritating materials from a portion of the Category III materials and then from the Category IV materials. A conservative cut-off value between the least irritating of the Category III materials and the Category IV materials seemed to be at MRD_{50} >80 mg/ml. This is slightly above the highest Category III value and in fact covers a significant number of the Category IV materials. Thus a material with an MRD_{50} score >80 mg/ml would be considered to have Category IV (there is only one example in this data set; all the other Category IV's would be overpredicted). Materials with MRD_{50} values between 80 mg/ml and 2 mg/ml would be considered to be Category III materials. Materials with MRD_{50} values ≤2 mg/ml would be considered to be Category I materials unless there was a second *in vitro* test which validated to categorize these materials into a I, II, or III category. Figure 6.1.3.5.c shows the 76 data points and the cut-off values represented by horizontal lines at MRD_{50} = 80 mg/ml and MRD_{50} =2 mg/ml.

For all three of the scoring systems the data distribution of the Company # 1 materials seems slightly different from that observed with data from the EC/HO, CTFA and COLIPA validation studies (Section 6.1.3.4). In general, the distribution is shifted to the milder end of the irritation scale. This is likely due to a combination of two phenomena: 1)

the Company #1 data set contains many marketed or prototype products which would likely be in the lower range of irritancy than materials in a validation study which are chosen to cover a wide range of the irritancy spectrum, and 2) the Company # 1 data have hazard classifications assigned based to ocular changes observed during a Low Volume Eye Test assay (10 µl of test material instilled in the eye) as opposed to the use of the traditional Draize test (100 µl of test material instilled in the eye) for hazard category determination that was used in the validation studies. It is known that the LVET does give somewhat lower irritation scores than does the traditional Draize test, although the LVET response is still generally more severe than the human response (Freeberg, Nixon et al. 1986) (Cormier, Parker et al. 1996).

The results of using the suggested cut-offs for the EU, GHS and EPA scoring systems are depicted in the contingency tables 6.1.3.5.b, 6.1.3.5.c and 6.1.3.5.d, respectively.

Table 6.1.3.5.b Company 1 - Contingency table depicting the concordance and predictivity of the CM assay for EU hazard classifications when the cut-off values from Figure 6.1.3.5.a are applied. N = 76 materials.

Draize Determined EU Category	EU Category Predicted by CM						
	R41	R36	Not Classified	Total	Concordance	Toxicity Overpredicted	Toxicity Underpredicted
R41	9		0	9	100.0%	NA	0%
R36	8		0	8	100.0%	NA	0%
Not Classified	43		16	59	27.1%	72.9%	NA
Total	60		16	76	43.4%		
Predictivity	28.3%		100.0%				
Category Underpredicted	NA		0%				
Category Overpredicted	71.7%		NA				

Table 6.1.3.5.c Company 1 - Contingency table depicting the concordance and predictivity of the CM assay for GHS hazard classifications when the cut-off values from Figure 6.1.3.5.b are applied. N = 76 materials.

Draize Determined GHS Category	GHS Category Predicted By CM							
	1	2A	2B	No Label	Total	Concordance	Toxicity Overpredicted	Toxicity Underpredicted
1	8		0	0	8	100.0%	NA	0%
2A	14		0	0	14	0%	100.0%	0%
2B	15		1	0	16	6.3%	93.8%	0%
No Label	23		8	7	38	18.4%	81.6%	NA
Total	60		9	7	76	39.5%		
Predictivity	36.7%		11.1%	100.0%				
Category Underpredicted	NA		0%	0%				
Category Overpredicted	63.3%		88.9%	NA				

Table 6.1.3.5.d Company 1 - Contingency table depicting the concordance and predictivity of the CM assay for EPA hazard classifications when the cut-off values from Figure 6.1.3.5.c are applied. N = 76 materials.

Draize Determined EPA Category	EPA Category Predicted By CM							
	I	II	III	IV	Total	Concordance	Toxicity Overpredicted	Toxicity Underpredicted
I	9		0	0	9	100.0%	NA	0%
II	10		0	0	10	100.0%	NA	0%
III	38		7	0	45	15.6%	84.4%	0%
IV	3		8	1	12	8.3%	91.7%	NA
Total	60		15	1	76	35.5%		
Predictivity	31.7%		46.7%	100.0%				
Category Underpredicted	NA		0%	0%				
Category Overpredicted	68.3%		53.3%	NA				

Table 6.1.3.5.e Company 1 - Concordance table for surfactant and surfactant containing materials for severe irritants versus the rest and non-irritants versus the rest for Company # 1 study. Cut-off values from Figures 6.1.3.5.a, 6.1.3.5.b, and 6.1.3.5.c were applied. N = 76 materials.

	Vs Rest													
	Concordance		Sensitivity		Specificity		Positive Predictivity		Negative Predictivity		False Positive Rate		False Negative Rate	
	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.
EU Severe irritants	33%	25/76	100%	9/9	24%	16/67	15%	9/60	100%	16/16	76%	51/67	0%	0/9
EU non-irritants	43%	33/76	100%	17/17	27%	16/59	28%	17/60	100%	16/16	73%	43/59	0%	0/17
GHS Severe irritants	32%	24/76	100%	8/8	24%	16/68	13%	8/60	100%	16/16	76%	52/68	0%	0/8
GHS non-irritants	59%	45/76	100%	38/38	18%	7/38	55%	38/69	100%	7/7	82%	31/38	0%	0/38
EPA Severe irritants	33%	25/76	100%	9/9	24%	16/67	15%	9/60	100%	16/16	76%	51/67	0%	0/9
EPA non-irritants	86%	65/76	100%	64/64	8%	1/12	85%	64/75	100%	1/1	92%	11/12	0%	0/64

An analysis of the ability of the CM assay to test surfactants and surfactant-based materials and separate severe irritants from "the rest", and non-irritants from "the rest" is shown in Table 6.1.3.5.e. The CM test seemed to perform best when identifying non-irritants – the negative predictive value was 100% for all the analysis conditions with only the EPA non-irritant analysis being somewhat suspect since there was only one negative material in the data set.

6.1.4 Compilation of data on predictive capacity of the test method

6.1.4.1 Description & rational for the prediction model(s) applied and statistical approaches used

EC/HO (Balls, Botham et al. 1995)

The original analysis of this study was conducted independently by BIBRA (Lovell) with guidance from the management team. There was no prediction model proposed before the start of the study. Post hoc analysis was conducted mainly by determining the Pearson product moment correlation between the *in vitro* score (MRD_{50}) and the Draize Modified Maximum Average Score (MMAS). A second comparison was made to chemicals having MMAS >59 and <59 and chemicals having MMAS >25 and <25 using the Mann-Whitney test. Subgroups of chemicals (solids, liquids, surfactants) were also analyzed. The subsets into which the chemicals fell were determined by the Chemicals Selection Committee. No special measures were taken to account for the uncertain solubility of some of the materials observed by some of the laboratories. The statistical analysis on these data examined the between-laboratory reproducibility of the method and the relationship between the SM with the transwell MRD_{50} values and *in vivo* data (presented as the MMAS score). After the analysis was concluded, it was determined that the CM with the transwell was not a valid assay for predicting the MMAS of either the universe of chemicals or any of the subgroups. In fact, none of the assays participating in the study were found to be valid for predicting the MMAS.

In the analysis conducted in this BRD, the CM with the transwell data were investigated for their ability to predict EU, GHS, or EPA hazard classifications. It appears that many R41, GHS Category 1, and EPA Category I materials – especially surfactants - can be distinguished from less irritating materials by using a cut-off or MRD_{50} < 2 mg/ml. The final sample size (11 materials) was very small, however.

CTFA Phase III (Gettings, Lordo et al. 1996)

The original analysis for this study was conducted by an independent contractor (Battelle Laboratories) using the SAS system for personal computers. The system performed a statistical analysis in three areas. The first area examined was the distributional characterization of the Draize and *in vitro* results. Next, the data obtained was analyzed for concordance with the Draize MAS (although only continuously distributed endpoints were measured). Regressional modeling of *in vitro* vs. MAS data was the ultimate method of analysis for this study since the main goal of this work was to predict Draize results based on *in vitro* information. The variability of the Draize results was estimated during this study to enable evaluation of the degree of separation between pairs of test materials. No attempt was made at the conclusion of the study to determine if any of the assays were actually valid for the purpose of predicting Draize MAS scores.

However, the CM was one of the better performing assays for these surfactants and surfactant-based formulations.

For this BRD, the relationship between MRD_{50} values and EU, GHS, and EPA hazard classifications was investigated. It was found that a cut-off value of >80 mg/ml might identify some Not Classified or No Category materials. However, there was much overlap between the other hazard categories.

COLIPA (Brantom, Bruner et al. 1997)

An aim of this study was the development and validation of a prediction model (PM) for each assay. Parallel *in vitro* and *in vivo* data generated in house, much of which had previously been voluntary submitted to IRAG (US Interagency Regulatory Alternatives Group), were evaluated to create a semi-logarithmic plot of the MRD_{50} vs. Draize MMAS for 133 surfactant products. A mathematical description of this plot served as the prediction model. BIBRA International performed a quality check on the data submitted by independent laboratories to determine if data generated in the study matched the prediction model. The CM PM for this study was a 3 parameter logistic model.

The formula proposed to relate *in vitro* MRD_{50} volumes and *in vivo* MMAS scores was:

$$MMAS = \frac{A}{1 + eB^{*} (\log10\ MRD_{50} - G)}$$

where A =148.0, B =1.813 and G =2.329

In addition, an *in vitro* cut-off score was proposed to provide a means of assessing the *in vitro* test data independent of any assumptions regarding the fit of the statistical methods to historical data. After the analysis, the CM using the proposed prediction model was not considered to be valid for the purpose of predicting Draize MAS scores.

In this BRD, a comparison was made to EU, GHS, and EPA hazard categories. It was clear from observations of the scatter plots that it would not be possible to set any cut-offs that would correctly identify test materials of intermediate, i.e. R36 or Category 2A or 2B materials. Therefore, an attempt was made to determine cut-off values that would identify either the more highly irritating materials or the non-irritating materials. Although It did not seem to be possible to adequately separate the non-irritating materials from the mid-level materials, a prediction model is proposed where, for surfactants, an MRD_{50} >10 mg/ml (for the GHS and EU systems), or an MRD_{50} > 80 mg/ml (for the EPA system) can identify Not Classified, No Category, or category IV materials, and an MRD_{50} <2 mg/ml can identify R41 or Category 1 materials. The prediction model was empirically determined by applying various cut-off values and calculating the number of either highly toxic or non-toxic substances that would be either under or overpredicted. A conservative approach was applied that attempted to minimize the number of under predictions.

Combined Studies

Data for surfactants and surfactant-containing products from the three studies mentioned above were combined to provide a much larger data set for analysis. This was done with knowledge of the fact that some of the materials used in the studies might be identical; however, it is not certain that the materials are identical. It was clear from observations of the scatter plots that it would not be possible to set any cut-offs that would correctly identify test materials of intermediate, i.e. R36 or Category 2A or 2B materials. Therefore, an attempt was made to determine cut-off values that would identify either the more highly irritating materials or the non-irritating materials. It was found that a prediction model could be proposed where, for surfactants and surfactant-containing materials, an MRD_{50} >10 mg/ml (for the GHS and EU systems), or an MRD_{50} >80 mg/ml (for the EPA system) can identify Not Classified or No Category materials, and an MRD_{50} <2 mg/ml can identify R41 or Category 1 or EPA Category IV materials.

Company # 1 Unpublished

The Company # 1 unpublished data analysis was slightly different than the preceding studies in that the Low Volume Eye Test was used for a standard rather than the traditional Draize test. It appeared from the scatter plots (Figures 6.1.3.5.a, 6.1.3.5.b and 6.1.3.5.c) that it was impossible to determine a cut-off value that would separate either the Category 1, 2A, or 2B categories from each other, the R41 and R36 categories from each other, or the EPA Category I, II, or III from each other. Therefore, an attempt was made just to identify a cut-off that would separate a high proportion of the No Label or No Category materials from the irritating materials. A conservative approach was taken where we sought to have as few under predictions as possible. The data in these studies suggested that – for surfactants and surfactant-containing products - a prediction model of MRD_{50} ≥10 mg/ml can be used to identify EU Not Classified or GHS No Category materials, and a prediction model of MRD_{50} ≥ 80 mg/ml can be used to identify EPA Category IV materials. These cut-offs were chosen to be somewhat higher than any of the MRD_{50} scores obtained by the R36, 2B or category III materials to give a higher probability that subsequent testing of a larger number of materials would not uncover any R36 or Category 2B or EPA category III materials that exceeded these limits.

However, more aggressive cut-off values of MRD_{50} ≥ 1 mg/ml for identifying EU No Label materials and GHS No Category materials were also suggested even though these values are very close to the less toxic R36 and Category 2B materials.

The distribution of the Company # 1 unpublished data points appears different than what was found for the other sets of data presented. This is likely due to the fact that the EU, GHS and EPA hazard categories were determined using the LVET rather than the traditional Draize assay. It is known that the LVET assay is gives somewhat lower classifications than does the Draize test, although both still over predict the human response.

6.1.4.2 Description of performance compared to reference and eventually, to the human situation for each study

EC/HO (Balls, Botham et al. 1995)

An analysis of the EC/HO data indicated that cut-offs could be applied which would have value in separating several of the hazard classes. The prediction model proposed was MRD_{50} > 80 mg/mL = Not Classified, No Label, and Category IV and MRD_{50} < 2 mg/mL = R41, Category 1, and Category I for the EU, GHS, and EPA classification systems respectively. The results of applying these prediction models to the data are shown in Tables 6.1.3.1.c, 6.1.3.1.d, and 6.1.3.1.e. The analysis of the surfactant materials only lead to the same conclusion for the prediction model as stated above. The results of applying these prediction models to the surfactant data are shown in Tables 6.1.3.1.g, 6.1.3.1.h, and 6.1.3.1.i.

CTFA Phase III (Gettings, Lordo et al. 1996)

An analysis of the CTFA data indicated that cut-offs could be applied which would have value in separating several of the hazard classes. The prediction model proposed was MRD_{50} > 80 mg/mL = Not Classified, No Label, and Category IV and MRD_{50} < 2 mg/mL = R41, Category 1, and Category I for the EU, GHS, and EPA classification systems respectively. The results of applying these prediction models to the data are shown in Tables 6.1.3.2.b, 6.1.3.2.c, and 6.1.3.2.d.

COLIPA (Brantom, Bruner et al. 1997)

An analysis of the COLIPA data indicated that cut-offs could be applied which would have value in separating several of the hazard classes. The prediction model proposed was MRD_{50} > 80 mg/mL = Not Classified, No Label, and Category IV and MRD_{50} < 2 mg/mL = R41, Category 1, and Category I for the EU, GHS, and EPA classification systems respectively. The results of applying these prediction models to the data are shown in Tables 6.1.3.3.b, 6.1.3.3.c, and 6.1.3.3.d. The prediction model proposed for the surfactant and surfactant containing materials was MRD_{50} > 10 mg/mL = Not Classified, and No Label, MRD_{50} > 80 mg/mL = Category IV and MRD_{50} < 2 mg/mL = R41, Category 1, and Category I for the EU, GHS, and EPA classification systems respectively. The results of applying these prediction models to the surfactant and surfactant containing materials are shown in Tables 6.1.3.3.e, 6.1.3.3.f, and 6.1.3.3.g.

Combined Studies

An analysis of the data for surfactant and surfactant-containing materials from the combined studies (EC/HO, CTFA Phase III and COLIPA) indicated that cut-offs could be applied which would have value in separating several of the hazard classes. The

prediction model proposed was MRD_{50} >10 mg/ml = Not Classified and No Label, and MRD_{50} <2 mg/ml = R41 and Category 1for the EU and GHS classification system respectively. The proposed prediction model for the EPA classification system was MRD_{50} > 80 mg/mL = Category IV and MRD_{50} < 2mg/mL = Category I. The results of applying these prediction models to the data are shown in the following contingency tables 6.1.3.4.b, 6.1.3.4.c, and 6.1.3.4.d

Because these contingency tables for the combined studies are based on the results with 53 test materials, they are a much better representation of the performance of the CM test than contingency tables based on any of the individual studies alone where far fewer materials were tested.

Company 1 Unpublished

Applying the prediction model of MRD_{50} > 10 mg/ml to the actual data in Figure 6.1.3.5.b reveals that 7 of the 38 materials (18.4%) identified as No Label by the animal test would be identified as No Label by the CM. This means that 81.6% of the animal designated No Category materials would have to pass on to a second level test.

If a more aggressive prediction model is used, e.g. MRD_{50} >1 is applied to Figure 6.1.3.5.b, then there would be significant improvement of the performance. Eighteen of the 38 materials (47.4%) identified as No Category by the animal test would be identified as No Label by the CM. However, one 2B material would now be under predicted by the model. Again, the remaining materials, 38 in this case, would have to pass on to a second level test in order to be correctly categorized.

An analysis of the data for surfactant and surfactant-containing materials from the Company # 1 study indicated that cut-offs could be applied which would have value in separating several of the hazard classes. The prediction model proposed was MRD_{50} >2 mg/ml = Not Classified and MRD_{50} <2 mg/ml = R41 for the EU classification system. The prediction model proposed was MRD_{50} >10 mg/ml = No Label and MRD_{50} <2 mg/ml = Category 1 for the GHS classification system. The prediction model proposed was MRD_{50} >80 mg/ml = Category IV and MRD_{50} <2 mg/ml = Category I for the EPA classification system. The results of applying these prediction models to the data are shown in the following contingency tables 6.1.3.5.b, 6.1.3.5.c, and 6.1.3.5.d

The above contingency tables indicate that a prediction model can be developed which allows little under prediction of the hazard categories of any of the classification systems, but it does not allow discrimination between the higher categories of irritancy.

A further analysis of the ability of the CM assay to categorize materials into two binary classification systems (severe irritants vs. the rest and non-irritants vs. the rest) is shown in Table 6.1.3.5.e.

6.1.4.3 Discussions

Description of the limitations of the test method (applicability domain based on the results of the data compilation)

Indications of the limitations of the test method first appeared in the EC/HO study. As seen in Tables 6.1.3.1.a and 6.1.3.1.b, R41 and Category I substances had a very wide range of CM MRD_{50} scores, ranging from approximately 0.3 mg/ml to nearly 100 mg/ml. The higher MRD_{50} values in this range overlapped significantly the No Label or No Category substances. These high scoring materials were of mixed chemistry – some with high pH (10 % sodium hydroxide), others of mixed chemistry (cyclohexanol, imidazole, pyridine, and 2,5-dimethyllohexanediol). By limiting the analysis to surfactants, Figures 6.1.3.1.d, 6.1.3.1.e, and 6.1.3.1.f show that most of the R41 and R36 with high MRD_{50} values as well as the Category 1 and 2A substances with high MRD_{50}'s were removed such that almost all the R41 and Category 1 materials had MRD_{50}'s <2 mg/ml. This was good evidence that the CM methodology was not very accurate for a broad range of chemical classes.

Subsequently the CTFA Phase III study showed that the 10 R41 surfactants or surfactant-containing materials and 8 Category 1 materials also had MRD_{50} values in the lower range, generally < 3 mg/ml.

Finally in the COLIPA there were several 2A and R36 non-surfactants which had relatively non-toxic MRD_{50} values. When the analysis was limited to surfactants, the separation by the cut off values seemed much clearer.

Thus, there is relatively strong evidence that the applicability domain of the CM assay is surfactants and surfactant-containing formulations. Although the data from Company # 1 do not add evidence against testing non-surfactant materials with the CM (since all the Company # 1 materials were surfactants) the studies do support the conclusion that non-irritant surfactant materials can be identified by their relatively high MRD_{50} values.

An additional part of the applicability domain was not really proven by the data of these studies but instead by the physical constraints of the test system, i.e. a certain number of materials were automatically excluded by the limitations of the machine itself. Any materials which are not completely water-soluble cannot be properly delivered by the pumping mechanisms of the machine and hence cannot even enter into the testing phase.

Possible rational(s) for differences observed

Apparent differences in the performance of the assay over the three major studies reviewed above are likely due to differences in the spectrum of surfactants or surfactant-containing materials that were tested, rather than differences in the CM's response to surfactants in general. This spectrum had an effect in the individual studies because so

few materials were tested in each one. A small variation in the irritation spectrum of the materials would likely make it appear that the prediction model might be different among the studies, yet when the studies were combined it was obvious that the hazard categories of the surfactants could be reasonably identified (at least the most toxic and least toxic of them) by high and low cut-off values.

6.2 Additional studies where raw data are not available

6.2.1 Relevant information for each study where raw data are not available

Table 6.2.1 Table presenting the relevant information for each study where raw data are not available

Studies	No. of chemicals	No. of products	Coded	No. of labs	No. of exps	Data format (raw, summary)	Chemical classes covered	Ranges of toxicity covered	Physico-chemical properties covered
Company # 1[1](Bruner, Miller et al. 1991)	4	13	Unknown	1	1	Summary	Household and personal cleaning products and ingredients	MAS 0-44.7	Liquids and granular materials
Several companies[2] (Bagley, Bruner et al. 1992)	12	20	Unknown	2	1	Summary	Cleaning product ingredients- almost all surfactants	MAS 0.3–43 for analysis; 0–44.7 total	See Annex A and B
Company # 2[3] (Catroux, Rougier et al. 1993)	21	32	Unknown	1	3	Summary	Surfactants and formulations	MAS 1.67-54	Liquids and water-soluble materials

[1] Performed using the silicon microphysiometer protocol with a 320-second exposures and normal human epidermal keratinocytes (see (Bruner, Miller et al. 1991)

[2] Performed using the silicon microphysiometer with glass coverslip standard protocol (as in 3) and the silicon microphysiometer with transwells with a 500-second exposure cycle

[3] Performed using the silicon microphysiometer instrument (transwell) with the 400-second exposure cycle

6.2.2 Relevant information for *in vivo* study where raw data are available

Table 6.2.2 Table presenting the *in vivo* reference data available

Studies	Species and protocols used as reference data	Sources of information	No. of labs	Quality of the reference data	Format of data available
Company # 1[1] (Bruner, Miller et al. 1991)	Albino rabbits using Low Volume Eye Test. No anesthesia. Whether every chemical was tested this way could not be determined	Previous testing	multiple	Not stated	Final LVET score only; 3-12 rabbits per study
Several companies[2] (Bagley, Bruner et al. 1992)	Albino rabbits using three different protocols. 1) Draize protocol with ocular anesthesia. 2) Draize protocol without ocular anesthesia, and 3) LVET protocol without anesthesia.	Not stated	At least 5.	Not stated	Single MAS value per chemcial
Company # 2[3] (Catroux, Rougier et al. 1993)	Rabbits tested using the Journal Officiel, 24 October 1984	Internal data bank	Unknown	Not stated	Summary MAS and MRD_{50} values in publication. More complete data for 20 of the 53 materials obtained from Company 2 for this BRD

[1] Performed using the silicon microphysiometer protocol with a 320-second exposures and normal human epidermal keratinocytes (see (Bruner, Miller et al. 1991)

[2] Performed using the silicon microphysiometer with glass coverslip standard protocol and the silicon microphysiometer with transwells with a 500-second exposure cycle

[3] Performed using the silicon microphysiometer instrument (transwell) with the 400-second exposure cycle

6.2.3. Brief description of the studies without available raw data

There were three studies which did not have raw data available. A comparison among the studies is somewhat difficult because different *in vitro* protocols were used in each study.

6.2.3.1 Company # 1 study (Bruner, Miller et al., 1991)

This study used the original SM instrument with normal human epidermal keratinocytes grown on a glass coverslip. Exposure was for approximately 300 seconds per dose. Seventeen materials were tested, 3 pure surfactants and 14 surfactant-containing formulations. LVET MAS scores were provided for each test substance along with the SEM and the number of rabbits used in the study (3-12).

6.2.3.2 Multiple lab study (Bagley, Brunner et al., 1992)

This study used two different SM protocols, one with L929 cells plated on glass coverslips and exposed for approximately 500 sec to each dose of test substance. The second protocol used the SM fitted with a transwell. L929 cells were grown on the membrane which formed the base of the transwell, and they were exposed to each dose for approximately 500 seconds, as in the first protocol. The purpose for using the two protocols was to compare data from the original SM with data from a new machine configuration that was to form the basis of a new commercial instrument, the CM.

Although there were 32 test materials studied in the project, only 17 were used for *in vivo*/*in vitro* comparisons since they all were tested with a traditional Draize test. The other materials were not compared because they were tested with the LVET. The materials were mostly surfactants and surfactant-containing formulations although a few were other ingredients often found in personal care or household cleaning products.

6.2.3.3 Company # 2 study (Catroux, Rougier et al. 1993)

This study used a SM fitted with a transwell chamber. L929 cells were grown in the chamber and exposed to test material for approximately 400 seconds per dose. Fifty-three materials were tested – 21 surfactants and 32 surfactant-based formulations. Draize tests were conducted on each material according to French legislation (Journal Officiel, 24 October 1984). Only summary Draize MAS scores were given in the manuscript, but more detailed mean date for rabbit groups was obtained from Company # 2 for some of the materials. However, since these additional data were group averages only and not individual animal scores, EH or GHS hazard categories could not be calculated.

6.2.4 Compilation of data on predictive capacity of the test method

6.2.4.1 Description & rational for the Prediction Model(s) applied and statistical approaches used

Company # 1 study

To analyze the performance of the SM, a semilog scatter plot was constructed between the mean and SEM of the MRD_{50} (in g/ml) and the LVET MAS plus SEM. Spearman's rank correlation test was then used to compare the relationship between the two values. No prediction model was proposed before the study was started.

Multiple lab study

Similar to the Company # 1 study, semilog scatter plots were constructed from the MRD_{50}'s from each SM protocol and LVET MAS values. Pearson and Spearman correlation coefficients were then generated to determine the strength of the relationship between the SM values and the LVET data. No prediction model was proposed before the study was started.

Company # 2 study

Several types of analyses were conducted to describe the performance of the SM instrument. One method was to construct a semilog scatter plot of MAS scores and MRD_{50} values for each of the test materials. A second method was to divide the MAS scale in to three classes (0-20, 20-40, and 40-60) and the log MRD_{50} into three similar classes (2.5-3.5, 3.5-5.0, and 5.0-6.5). Data points falling within each corresponding "box" were considered to be correct predictions. Data points falling outside their respective "boxes" were considered to be under or over predictions as appropriate. No prediction model was proposed before the study was started.

6.2.4.2 Description of performance compared to reference and eventually, to the human situation for each study

Company # 1 study

The SM data were compared to the LVET MAS scores on a semilog plot. Spearman's rank correlation test showed an r value of 0.89 between the two values indicating a relatively good predictive value of the SM method for the rabbit score. Only one material – Hard Surface Cleaner B – was a clear outlier, and it was under predicted.

Multiple lab study

Both the SM with a glass coverslip and the SM with a transwell chamber were compared to the LVET MAS for 17 substances. For the glass coverslip method the

Pearson/Spearman correlation coefficients were -0.71 and -0.65, respectively. For the SM with transwell chamber the coefficients were -0.72 and -0.61, respectively. Thus both methods predicted the animal score reasonably well. An examination of the scatterplots shows that MAS values <10 had log $1/MRD_{50}$ values which extended over at least two logs. The curve then rose steeply over the next two logs to the maximum tested LVET scores of slightly less than 50.

Company # 2 study

Comparisons were first made directly between the MAS score and the log MRD_{50}. Pearson and Spearman coefficients were calculated for the 53 test materials and were found to be 0.91 and 0.89 respectively. This indicates a very good predictive capacity of the SM for the Draize MAS scores up to about MAS=54.

A second analysis was conducted by dividing the scores into three classes each. This analysis showed that there were 8 false positives and no false negatives. This type of analysis is not very common since there is little chance for a correct prediction at points where "correct" boxes are adjacent. This is because the boxes only touch at a corner (essentially a point) and any scatter of points at all would create many outliers. None the less, this analysis indicates that there is quite a good predictive power to the SM assay as used here.

Several types of analyses were conducted to describe the performance of the SM instrument. One method was to construct a semilog scatter plot of MAS scores and MRD_{50} values for each of the test materials. A second method was to divide the MAS scale in to three classes (0-20, 20-40, and 40-60) and the log MRD_{50} into three similar classes (2.5-3.5, 3.5-5.0, and 5.0-6.5). Data points falling within each corresponding "box" were considered to be correct predictions. Data points falling outside their respective "boxes" were considered to be under or over predictions as appropriate.

6.2.4.3 Discussions

All three of the studies used similar test substances – almost all surfactants or surfactant-containing formulations. Even though the protocols varied somewhat – mouse cells versus human cells, cells grown on cover slips versus cells grown in transwell chambers, and different exposure times all studies showed good prediction of the ocular irritation level (as measured by the MAS score) of the test substances.

6.3 Attempt to combine the data using weigh-of-evidence approaches

All three studies reported here were very similar in their construction, even though details of their protocols varied. Each tested very similar materials (surfactants and surfactant containing formulations). Only one test (Bagley, Bruner et al. 1992) was reported as being conducted on coded materials, but the automated data recording attributes of the SM tend to eliminate most bias that might occur by knowing the identity of

the test material. Since the reported Pearson's correlation coefficient for all of the four methods was >0.82 (individual r values were 0.97, 0.82, 0.86, and 0.91) the three studies support each other very well and give weight to the conclusion from these studies that the SM (using any of a number of different protocols) predicts the rabbit MAS very well, between 0 and ~50.

7. Applicability Domain (Module 6)

Much of the definition of the applicability domain has been influenced by the physical constraints imposed by the SM or CM machine itself, or by in-house studies that have not been reported in the open literature. Although some of the information shown in this BRD supports the existing feeling that the domain of the SM or CM is for surfactants and water soluble surfactant-containing products, there is no overwhelming data to support this point.

Clearly the physical properties of the machine which require it to expose the cells by pumping test material through a small diameter tube, and then wash the cells by pumping fresh media across the cells and out the chamber through another small diameter tube, dictate that no solids or suspensions be used. Materials of this physical state would tend to clog the machine or not be washed out once they had reached the exposure chamber. Thus test substances should be limited to water-soluble materials.

Personal communication with users of the CM over the last decade indicate that their experience is that most non-surfactant substances are not well predicted by the CM. Conversely they feel that surfactants and surfactant containing materials are well predicted. There are not strong data in this BRD to support the view that non-surfactants are not well predicted since very few non-surfactant materials were tested. Only the rather poor predictive results from the EC/HO study which used many non-surfactant materials would support this view.

The general class of materials (and the irritancy level) that does seem to be reasonably predicted is mild surfactants and surfactant-containing formulations as shown by the COLIPA study, the CTFA study, and the internal Company # 1 data.

This highlights an important role for a test like the CM. It can be used to immediately identify very mild surfactant-containing materials which may be a useful feature for a cosmetics or personal care product company that desires to produce products at the very low end of the irritancy scale. If the material is more irritating than the cut-off level chosen for the very mild products, a second type of *in vitro* test having a more robust nature, e.g a three dimensional tissue or an ex vivo eye model, could be used to properly classify the material in one of the higher hazard classification levels.

8. Supporting materials

8.1 Relevant publications, other scientific reports and reviews

Araki, K., Y. Ohahsi, S. Knoshita, K. Hayahsi, Y. Kuwayama and Y. Tano (1994). "Epithelial Wound Healing in the Denervated Cornea." Current Eye Research **13**: 203-211.

Bagley, D. M., L. H. Bruner, O. De Silva, M. Cottin, K. A. O'Brien, M. Uttley, et al. (1992). "An Evaluation of Five Potential Alternatives In Vitro to the Rabbit Eye Irritation Test In Vivo." Toxicology In Vitro **6**(4): 275-284.

Baldwin, H. A., T. O. McDonald and C. H. Beasley (1973). "Slit examination of experimental animals eyes. II Grading scales and photographic evaluation of induced pathological conditions." J Society of Cosmetic Chemistry **24**: 181-195.

Balls, M., P. A. Botham, L. H. Bruner and H. Spielmann (1995). "The EC/HO International Validation Study on Alternatives to the Draize Eye Irritation Test." Toxicology In Vitro **9**(6): 871-929.

Botham, P., R. Osborne, K. Atkinson, G. Carr, M. Cottin and R. G. Van Buskirk (1997). "IRAG Working Group 3. Cell Function-based Assays. Interagency Regulatory Alternatives Group." Food and Chemical Toxicology **35**(1): 67-77.

Brantom, P. G., L. H. Bruner, M. Chamberlain, O. De Silva, J. Dupuis, L. K. Earl, et al. (1997). "A Summary Report of the COLIPA International Validation Study on Alternatives to the Draize Rabbit Eye Irritation Test." Toxicology In Vitro **11**: 141-179.

Bruner, L. H., D. J. Kain, D. A. Roberts and R. D. Parker (1991). "Evaluation of Seven In Vitro Alternatives for Ocular Saftey Testing." Fundam Appl Toxicol **17**(1): 136-149.

Bruner, L. H., K. M. Miller, J. C. Owicki, J. W. Parce and V. C. Muir (1991). "Testing ocular irritancy in vitro with the silicon microphysiometer." Toxicology In Vitro **5**: 277-284.

Butler, J. M. and B. R. Hammond (1980). "The Effects of Sensory Denervation on the Response of the Rabbit Eye to prostaglandin E1, bradykinin and substance P." British Journal of Pharmacology **69**: 495-502.

Catroux, P., A. Rougier, K. G. Dossou and M. Cottin (1993). "The Silicon Microphysiometer for Testing Ocular Toxicity In Vitro." Toxicol In Vitro **7**(4): 465-469.

Chan, P. K. and A. W. Hayes (1994). "Acute Toxicity and Eye Irritancy." In Principles and Methods of Toxciology, 3rd Edition (ed Hayes A.W.): 579-647.

Cormier, E. M., R. D. Parker, C. Henson, L. W. Cruze, A. K. Merritt, R. D. Bruce, et al. (1996). "Determination of the Intra- and Inter-Laboratory Reproducibility of the Low Volume Eye Test and its Statistical Relationship to the Draize Test." Reg. Tox. Pharmac. **23**: 156-161.

Dearman, R. J., M. Cumberbatch and I. Kimber (2003). "Cutaneous Cytokine Expression: Induction by Chemical Allergen and Paracrine Regulation." Journal of Toxicology-Cutaneous and Ocular Toxicology **22**: 69-86.

DeSousa, D. J., A. A. Rouse and W. J. Smolon (1984). "Statistical Consequences of Reducing the Number of Rabbits Utilized in Eye Irritation Testing: Data on 67 Petrochemicals." Toxicol Appl Pharmacol **76**: 234-242.

Dua, H. S., J. A. P. Gomes and A. Singh (1994). "Corneal Epithelium Wound Healing." British Journal of Ophthamology **78**: 401-408.

ECETOC (1992). "ECETOC Technical Report No 48."

ECETOC (1998). "ECETOC Technical Report No. 48 (2)."

Eurell, T. E., J. M. Sinn, P. A. Gerding and C. L. Alden (1991). "In Vitro Evaluation of Ocular Irritatns Using Corneal Protein Profiles." Toxicology and Applied Pharmacology **108**: 374-378.

Farquhar, M. G. and G. E. Palade (1963). "Junctional complexes in Various Epithelia. ." J Cell Biology **17**: 375-412.

Feder, P. I., R. A. Lordo, D. M. B. Dipasquale, M. Chudkowski, J. L. Demetrulias, K. L. Hintze, et al. (1991). "The CTFA Evaluation of Alternatives Program: An Evaluation of *In Vitro* Alternatives to the Draize Primary Eye Irritation Test (Phase I) Hydro-alcoholic Formulations; (Part 1) Statistical Methods." In Vitro Toxicology **4**(4): 231-246.

Freeberg, F. E., G. A. Nixon, P. J. Reer, J. E. Weaver, R. D. Bruce, J. F. Griffith, et al. (1986). "Human and Rabbit Eye Responses to Chemical Insult." Fund. Appl. Toxic. **7**: 626-634.

Gautheron, P., J. Giroux, M. Cottin, A. Audegond, A. Morilla, L. Mayordomo-Blanco, et al. (1994). "Interlaboratory Assessment of the Bovine Corneal Opacity and Permeability (BCOP) Assay." Toxicology In Vitro **8**(3): 381-392.

Gettings, S. D., L. C. Dipasquale, D. M. Bagley, P. L. Casterton, M. Chudkowski, R. D. Curren, et al. (1994). "The CTFA Evaluation of Aternatives Program: An Evaluation of *In Vitro* Alternatives to the Draize Primary Eye Irritation Test. (Phase II) Oil/Water Emulsions." Fund. Chem. Toxic. **32**(10): 943-976.

Gettings, S. D., R. A. Lordo, K. L. Hintze, D. M. Bagley, P. L. Casterton, M. Chudkowski, et al. (1996). "The CTFA Evaluation of Alternatives Program: an evaluation of in vitro alternatives to the Draize primary eye irritation test. (Phase III) surfactant-based formulations." Food Chem Toxicol **34**(1): 79-117.

Griffith, J. F., G. A. Nixon, R. D. Bruce, P. J. Reer and E. Bannan (1980). "Dose response studies with chemical irritants in albino rabbits as a basis for selecting optimum testing conditions for predicting hazard to the human eye." Toxicology and Applied Pharmacology **55**: 501-513.

Hackett, R. B. and T. O. McDonald (1994). "Mechanisms of Ocular Response to Irritants." Dermatotoxicology, 5th Editions, (ed Marzulli F.N, Maibach H.I.): 299-306.

Harbell, J., S. W. Koontz, R. W. Lewis, D. Lovell and D. Acosta (1997). "IRAG Working Group 4: Cell Cytotoxicity Assays." Food and Chemical Toxicology **35**: 79-126.

Harbell, J. W., R. Osborne, G. J. Carr and A. Peterson (1999). "Assessment of the Cytosensor™ Microphysiometer Assay in the COLIPA In Vitro Eye Irritation Validation Study." Toxicology In Vitro **13**: 313-323.

Hartung, T., S. Bremer, S. Casati, S. Coecke, R. Corvi, S. Fortaner, et al. (2004). "A Modular Approach to the ECVAM Principles on Test Validity " Atern Lab Anim **32**: 467-472.

Hogan, M. J. and L. E. Zimmerman (1962). "Ophthalmic Pathology: An Atlas and Textbook, 2nd edition." Philadelphia: W.B. Saunders.

Hubert, F. (1992). "The Eye (Rabbit/Human): Parameters to be Measured in the Field of Ocular Irritation." ATLA **20**: 476-479.

Jacobs, G. A. and M. A. Martens (1990). "Quantification of Eye Irritation Based Upon In Vitro Changes of Corneal Thickness." ATLA **17**: 255-262.

Jester, J. V., L. Li, A. Molai and J. K. Maurer (2001). "Extent of initial corneal injury as a basis for alternative eye irritation tests." Toxicol In Vitro **15**(2): 115-30.

Jester, J. V., W. M. Petroll, J. Bean, R. D. Parker, G. J. Carr, H. D. Cavanagh, et al. (1998). "Area and depth of surfactant-induced corneal injury predicts extent of subsequent ocular responses." Invest Ophthalmol Vis Sci **39**(13): 2610-25.

Katahira, J. H., H. Sugiyama, N. Inoue, Y. Horiguchi, M. Matsuda and N. Sugimoto (1997). "Clostridium Perfringens Enterotoxin Utilizes Two Structurally Related Membrane Proteins as Functional Receptors in Vivo." J Biological Chemistry **272**: 26652-26658.

Klyce, S. D. and R. W. Beuerman (1988). "Structure and Function of the Cornea." The Cornea: 3-23.

Lovell, D. "EC/HO International Study on Alternatives to the Draize Eye Test." BIBRA Project No. 1367/1.

Maurer, J. K. and R. D. Parker (1996). "Light Microscope Comparision of Surfactant Induced Eye Irritation i Rabbits and Rats at Three Hours and Recovery/day 35." Toxicologic Pathology **24**: 403-411.

Maurer, J. K., R. D. Parker and J. V. Jester (2002). "Extent of initial corneal injury as the mechanistic basis for ocular irritation: key findings and recommendations for the development of alternative assays." Regul Toxicol Pharmacol **36**(1): 106-117.

Maurer, J. L. and R. D. Parker (1996). "LIght Microscope Comparison of Surfactant-induced Eye Irritation in Rabbits and Rats at Three Hours and Recovery/day 35." Toxicologic Pathology **6**: 464-477.

McConnell, H. M., J. C. Owicki, J. W. Parce, D. L. Miller, G. T. Baxter, H. G. Wada, et al. (1992). "The Cytosensor Microphysiometer: Biological Applications of Silicon Technology." Science **257**(5078): 1906-12.

Parce, J. W., J. C. Owicki, K. M. Kercso, G. B. Sigal, H. G. Wada, V. C. Muir, et al. (1989). "Detection of Cell-affecting Agents with a Silicon Biosensor." Science **246**: 243-247.

Prinsen, M. K. (2006). "The Draize Eye Test and in vitro alternatives; a left-handed marriage?" Toxicol In Vitro **20**(1): 78-81.

Van Meer, G., W. van Hof and I. nan Genderen (1992). "Tight Junctions and Polarity of Lipids " Tight Junctions (ed Cereijido M.): 187-201.

Wilhelmus, K. R. (2001). "The Draize Eye Test." Surveys in Ophthalmology **45**: 493-515.

National Toxicology Program
P.O. Box 12233
Research Triangle Park, NC 27709

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health